PCT / IB 04 / 02531

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International Application No. / Demande internationale n° PCT/IB 03 / 03861

International Filing Date / Date du dépôt international 18 AUGUST 2003 (18.08.03)

Geneva/Genève, 10 AUGUST 2004 (10.08.04)

International Bureau of the World Intellectual Property Organization (WIPO)

Bureau International de l'Organisation Mondiale de la Propriété Intellectuelle (OMPI)

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Head, PCT Receiving Office Section
Chef de la section "office récepteur du PCT"

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REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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PCT / IB 0 3 / 0 3 8 6 1
International Application No.

18 AUGUST 2003 (18.08.03)
International Filing Date

INTERNATIONAL BUREAU OF WIPO
PCT International Application
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)*

Box No. I TITLE OF INVENTION

Novel therapeutic extracts and molecules (Yezdex) for diabetes using Avestha's metabolite grid (Metagrid)

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Avestha Gengraine Technologies Pvt. Ltd
"Discoverer" 9th Floor, Unit 3
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Bangalore 560 066, India.

Telephone No.: +91-80-8411665
Facsimile No.: +91-80-8418780
Teleprinter No.: NA
Applicant's registration No. with the Office: NA

State *(that is, country)* of nationality: India

State *(that is, country)* of residence: India

This person is applicant for the purposes of: ☒ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Dr. Villoo Morawala Patell, Founder & CEO,
Avestha Gengraine TechnologiesPvt. Ltd.
"Discoverer" 9th Floor, Unit 3
International Tech Park, Whitefield Road,
Bangalore 560 066, India.

This person is:
☐ applicant only
☒ applicant and inventor
☒ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office: NA

State *(that is, country)* of nationality: India

State *(that is, country)* of residence: India

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☐ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Telephone No.
Facsimile No.
Teleprinter No.
Agent's registration No. with the Office

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. [8]. 2

Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

☐ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **MZ** Mozambique, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** United Republic of Tanzania, **UG** Uganda, **ZM** Zambia, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☐ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **BG** Bulgaria, **CH & LI** Switzerland and Liechtenstein, **CY** Cyprus, **CZ** Czech Republic, **DE** Germany, **DK** Denmark, **EE** Estonia, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **HU** Hungary, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **RO** Romania, **SE** Sweden, **SI** Slovenia, **SK** Slovakia, **TR** Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☐ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GQ** Equatorial Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

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☐ **AL** Albania .....
☐ **AM** Armenia .....
☐ **AT** Austria .....
☐ **AU** Australia .....
☐ **AZ** Azerbaijan .....
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☐ **CA** Canada
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☐ **CO** Colombia .....
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☐ **DK** Denmark .....
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☐ **GB** United Kingdom
☐ **GD** Grenada
☐ **GE** Georgia .....
☐ **GH** Ghana .....
☐ **GM** Gambia

☐ **HR** Croatia .....
☐ **HU** Hungary .....
☐ **ID** Indonesia
☐ **IL** Israel .....
☐ **IN** India .....
☐ **IS** Iceland
☐ **JP** Japan .....
☐ **KE** Kenya .....
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Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ ..... ☐ ..... ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Sheet No. [5] 3

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1)				
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* . . . .

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following **declarations** are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*: Number of declarations

☐	Box No. VIII (i)	Declaration as to the identity of the inventor	:
☐	Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐	Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☒	Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐	Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

Sheet No. [8] 4 A

Box No. VIII (iv) DECLARATION: INVENTORSHIP (only for the purposes of the designation of the United States of America)

The declaration must conform to the following standardized wording provided for in Section 214; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (iv). If this Box is not used, this sheet should not be included in the request.

Declaration of inventorship (Rules 4.17(iv) and 51*bis*.1(a)(iv)) for the purposes of the designation of the United States of America:

I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.

This declaration is directed to the international application of which it forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/..... (if furnishing declaration pursuant to Rule 26*ter*).

I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.

I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.

Prior Applications: .....

.....

I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

A RO

Name: Dr. Villoo Morawala Patell

Residence: Bangalore, Karnataka, India
(city and either US state, if applicable, or country)

Mailing Address: Avestha Gengraine Technologies Pvt. Ltd. " Discoverer" 9th Floor, Unit 3,
International Tech Park, whitefield Road, Bangalore 560 066, India.

Citizenship: Indian

Inventor's Signature: .....
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 11th Aug 2003
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

Name: .....

Residence: .....
(city and either US state, if applicable, or country)

Mailing Address: .....

.....

Citizenship: .....

Inventor's Signature: .....
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: .....
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)".

Sheet No. [12] 5

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application **contains:**

(a) **in paper form,** the following number of sheets:

request (including declaration sheets)	:	5 [12]
description (excluding sequence listings and/or tables related thereto)	:	29 [22]
claims	:	1
abstract	:	1
drawings	:	26 [34]
Sub-total number of sheets	:	62 [70]
sequence listings	:	
tables related thereto	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)		
Total number of sheets	:	62 [70]

(b) ☐ **only in computer readable form** (Section 801(a)(i))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

(c) ☐ **also in computer readable form** (Section 801(a)(ii))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the
- ☐ sequence listings: .....
- ☐ tables related thereto: .....

(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)

This international application is **accompanied by** the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item):*

	Number of items
1. ☐ fee calculation sheet	:
2. ☐ original separate power of attorney	:
3. ☐ original general power of attorney	:
4. ☐ copy of general power of attorney; reference number, if any:	:
5. ☐ statement explaining lack of signature	:
6. ☐ priority document(s) identified in Box No. VI as item(s):	:
7. ☐ translation of international application into *(language)*:	:
8. ☐ separate indications concerning deposited microorganism or other biological material	:
9. ☐ sequence listings in computer readable form *(indicate type and number of carriers)*	
(i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application)	:
(ii) ☐ *(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter*	:
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listings mentioned in left column	:
10. ☐ tables in computer readable form related to sequence listings *(indicate type and number of carriers)*	
(i) ☐ copy submitted for the purposes of international search under Section 802(b-*quater*) only (and not as part of the international application)	:
(ii) ☐ *(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-*quater*)	:
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:
11. ☐ other *(specify)*:	:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Dr. VILLOO MORAWALA - PATELL
FOUNDER & CEO
AVESTHA GENGRAINE TECHNOLOGIES PVT. LTD.
"DISCOVERER", 9th FLOOR
INTERNATIONAL TECHNOLOGY PARK
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BANGALORE - 560 066

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1. Date of actual receipt of the purported international application: 18 AUGUST 2003 (18.08.03)
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA / US
6. ☒ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

Novel therapeutic extracts and molecules (Yezdex) for Diabetes using Avestha's metabolite grid (*Metagrid*)

Field of the Invention

The present invention relates to a plethora of selected plants, which were isolated and characterized for the therapeutically relevant molecules to be used in the treatment of Diabetes. The extracts from the plants were subjected to both, targeted and non-targeted screening procedures. The ongoing-targeted screening procedures, which feature a comprehensive metabolite profiling of multitudes of phyto-extracts, were envisaged to facilitate the creation of a metabolite grid. Extensive comparative analyses of the individual plant species with the existing drug and/or phyto-extract formulations in the market has revealed the presence of both, unique and common molecular constituents that will be used individually and/or in combination to accelerate the process of the discovery of novel therapeutic formulations. This invention also relates to an edible composition comprising of fifteen Indian herbal extracts which can be used as a dietary supplement and also useful in lowering the glucose levels in the blood of mammals, particularly humans, suffering from Diabetes *mellitus*.

Background of the Invention

Diabetes is often defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and post-prandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to a condition known as hyperglycemia that will in due course turn into a syndrome called Diabetes *mellitus*. The word Diabetes is derived from the Greek work "Diab", meaning to pass through, namely, referring to the cycle of heavy thirst and frequent urination. *Mellitus* is derived from the Latin word "sweetened with honey" and alludes to the presence of sugar in the urine. Diabetes *mellitus* is the most common metabolic abnormality in the world. It is a metabolic disorder characterized by a lack of hormone insulin in the blood, which leads to abnormalities in the assimilation of carbohydrates in the body. Diabetes *mellitus* can be defined as a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins along with increasing risks of complication from vascular diseases. Very often, the seriousness of the disease is realized on the development of secondary symptoms that manifest in many forms, namely, difficulty in healing of wounds, neuropathy and so on. It is estimated currently, that over 143 million people all over the world suffer from Diabetes *mellitus* and in the case of most people, suffering from Diabetes; it is not properly diagnosed until irreversible complications set in.

Diabetes has been known to be prevalent in India since the past few centuries and early recorded literature has even documented the physician, Sushruta's description of Diabetes around 1,000 B.C. and later on the physician, Charak. It is in fact believed that the knowledge of the syndrome of Diabetes *mellitus*, existed in India since pre-historic age. Its earliest reference (1,000 B.C.) in the Ayurvedic literature is found in mythological form, where it is said to have originated by eating Havisha, a special

food that used to be offered at the time of Yagna organised by Daksha Prajapati (Kohli et al., Ayurvedic perspective of Diabetes and allied disorders, Vedic Life sciences).

However in developing countries like India, there is not much focus on Diabetes, given its chronic and slow nature. Moreover the economics of developing countries do not permit for devoting its resources to an understanding or search for remedies. The World Health Organisation has projected India, as the country with the fastest growing population of diabetics and it is estimated that between 1995-2025, Diabetes patients in India will increase by 195%.

Amongst the many classical, clinical symptoms associated with Diabetes, one which is typical, is an increase in the blood glucose, otherwise known as hyperglycemia, which in turn may result in polyuria (frequent urination), polydipsia (excessive thirst), polyphagia (excessive hunger), weight loss and blurred vision, apart from glycosuria and acetone breath. The long terms complications arising out of untreated or ineffectively treated Diabetes include among others, retinopathy, nephropathy and peripheral neuropathy. Diabetes patients stand an increased risk of succumbing to cardiovascular diseases and strokes.

Recent developments in understanding the pathophysiology of the disease process have opened up several new avenues to identify and develop novel therapies to combat the diabetic plague. Phytochemicals identified from traditional medicinal plants present an exciting opportunity to develop new kinds of therapeutics. There is an urgent need to identify indigenous natural resources, procure them and study them in detail and their potential on newly identified targets in order to develop them as new therapeutics.

The increasing cost of modern treatment of Diabetes indicates a great need for the development of alternate strategies for the prevention and treatment of Diabetes. In rural pockets of developing countries, almost 90% of the population still rely on traditional medicines for their primary health care and an investigation into such traditional medicines have led to the discovery of at least 88 drugs. There is a need for a rationally designed interdisciplinary research programme, which could lead to the development of indigenous, renewable medicinal plant sources as practical and cost effective alternatives. It is believed that the therapeutic approach of several traditional medicinal systems is more holistic. The medicinal preparations from traditional medicines contain a variety of herbal and non-herbal ingredients that are believed to act on a variety of targets through various modes and mechanisms.

Since ancient times, traditional medicines all over the world have advocated the use of plants to treat Diabetes. The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of beta cells, insulin releasing activity, improvement of the glucose uptake, utilisation and the anti oxidant properties present in the medicinal plants, which offers exciting opportunities, to develop them into novel therapeutics.

The multifactorial pathogenicity of Diabetes demands a multi – modal therapeutic approach. Thus, future therapeutic strategies require the combination of various types of multiple agents. *Medicatrix naturae* or the power of self-preservation or adjustment has been the motto of traditional medicinal

practise, which prescribes poly herbal formulations. The theories of poly herbal formulation have the synergistic, potentiative, agnoistic/antognistic pharmacological agents within themselves due to the incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles are known to work in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. Traditional medicines ought not to be treated as a collection of therapeutic recipes. They are formulated and prepared, keeping in mind, the disease/sickness and the healing properties of the individual ingredients.

In the case of Diabetes, the main symptoms targeted were thirst, polyuria and glycosuria (Herbal Drugs as Antidiabetics: An Overview; Sanjay M. Jachak, CRIPS Vol. 3, Number 2, April-June 2002). There have been over 1200 plants that have been screened for activity on the basis of ethnopharmacology or on a random basis. Plant extracts have been tested under specific conditions as glucose-loaded, alloxan, streptozotocin or naturally diabetic subjects in various animals like rodents.

The Indian subcontinent is renown for its indigenous collection of natural remedies like Ayurveda, Unani, Siddha and so on, based on which, new therapeutic molecules can be obtained. A large number of drugs from plant sources are secondary metabolites, which have no role in plant metabolism, but are expected to play a significant role in plant defence mechanism. There is however, not much difference seen between the basic metabolic processes in plants and animals. There are approximately around 200 pure compounds from plant sources, which are reported to indicate a decrease in the blood glucose.

Over 1200 plant compounds worldwide have undergone tests at various levels in order to ascertain their ability to lower blood sugar levels and it was found that many of these compounds contained chemical components that possessed hypoglycemic activity, when, tested in either animal models or test tubes. However the research on such compounds involving human subjects has not brought much information to light so far. There have been a few herbal remedies for Diabetes tested on humans and these have revealed mild blood sugar lowering properties.

Type II Diabetes also known as non-insulin dependent Diabetes *mellitus* ('NIDDM') is more common than insulin dependent Diabetes *mellitus*, affecting 80-90% of all people affected with Diabetes. Initially NIDDM is often of gradual onset during the latter middle age. Later stages of this disease are very severe resulting in long term complications like kidney failure, heart problems, eye and nervous disease and other diseases as well. Obesity is an important factor in NIDDM. The symptoms can be vague and include fatigue, nausea, frequent urination and unusual thirst. NIDDM develops genetically in predisposed individuals. The pathological changes in the pancreatic islets of patients with NIDDM are not always apparent. Many patients are known to have normal to high plasma insulin levels. In such cases, very often, Diabetes does not arise from a shortage of insulin, but as a result of defects in the molecular machinery that mediates the action of insulin on its target cells. NIDDM is also caused by destruction of other mechanisms such as insulin resistance, down-regulation of insulin receptors, defects in insulin secretion from the pancreatic cells and other changes to the glucose transporter system. To date, no satisfactory method exists to treat or cure NIDDM without apparent toxicity to the patients. Therefore there is an urgent need to provide alternative treatments, effective in the prevention and/or treatment of NIDDM.

An ideal medication for the treatment and prevention of Diabetes would be one which would incorporate the following characteristics: ability to stimulate regeneration of pancreatic islets and beta cells responsible for insulin production and to increase c-peptide levels; ability to modulate the autoimmune destruction of the cells responsible for insulin production; ability to correct the dislipidemia associated with Diabetes; ability to decrease insulin resistance, with few or no side effects. However the known pharmaceutical compositions for treating Diabetes do not meet with this criterion. The pharmaceutical oral hypoglycemic agents produce inconsistent clinical results with frequent, severe side effects and there is a need felt for safe and effective oral hypoglycemic agents that provide the clinician with a wide range of options to prevent, treat and manage Diabetes since it has been held that the traditional drugs prescribed to balance blood sugar can result in serious liver damage and in some cases, even cause liver failure. Although many traditional plant treatments for Diabetes exist, it is only a few that have received scientific or medical scrutiny (Gray & Flatt Insulin-secreting activity of the traditional anti diabetic plant Viscum album (mistletoe) – Journal of Endocrinology, 1999).

Herbs have been frequently alluded to as part of 'nature's pharmacy'. Even though herbal drugs act in more than one way analogous to modern drugs, herbal remedies are generally looked upon as a lot safer. In fact, many of the drugs used in conventional medicine are derived from herbs, instead of isolating the 'active agent'. Herbalism uses the whole plant. In many cases it has been observed that plants contain constituents that work together synergistically and using the whole plant aids in decreasing the side effects that may occur when using isolated components.

Plants with medicinal properties, the gift of Mother Nature to mankind, have been in use in India over several centuries in the traditional systems of medicine like Ayurveda, Unani etc., for the treatment of diseases including Diabetes *mellitus*. They are considered to be effective and toxic. It is botanical medicine that provides a complete system of healing and prevention of the disease. It is the oldest and most natural form of medicine. Its history of efficacy and safety spans centuries and covers almost the entire planet. Since herbal medicine is holistic medicine, it is able to look beyond the symptoms to the underlying systemic imbalance and offers real and permanent solutions to the problems on hand.

Higher plants constitute one of our most important natural resources. They not only provide foodstuffs, fibers, and woods, but many biochemicals, such as oils, flavorings, dyes, and pharmaceuticals. Although plants are renewable resources, some species are becoming more difficult to obtain in sufficient amounts to meet increasing demands. Destruction of natural habitats and technical difficulties in cultivation too are responsible for the drastic reduction in plant availability. For example, it is claimed that a demand for paclitaxel, a potent anticancer compound, could endanger the forests of *Taxus brevifolia* (Pacific yew) because of the low paclitaxel content (40–100 mg/kg of bark) in and a slow growth of the trees.

For many natural chemicals it is possible to synthesize alternatives from petroleum, coal, or both. The economic limitations of chemical synthesis and the pollution that accompanies this type of chemical synthesis, however, have led to the development of cell culture and molecular engineering of plants for the production of important chemicals. Plant cell and organ cultures offer promising alternatives for the production of biochemicals since totipotency enables plant cells and organs to produce useful secondary metabolites *in vitro*. Molecular engineering of secondary metabolites has the potential to increase productivity and improve product composition.

The metabolism comprises a coordinate series of coupled enzymatic conversion in living organisms. The secondary metabolites are not vital to the cell death that produces them but contribute to the overall fitness of the organisms. The functions of these compounds in plants include protection against pests and pathogens. For man, plant secondary metabolites are useful as pharmaceutical dyes, fragrance, insecticides and/or flavours.

In order to regulate the biosynthesis of secondary metabolites, plants must accommodate their primary metabolic pathways. A coordinate regulation between these processes has been observed but the regulatory mechanisms are unknown. (Lelslie van der Fits & Johan Memelink, 2000). Production of secondary metabolites is controlled at the levels of expression of the biosynthetic genes by developmental tissue specific factors or by external signals. The accumulation of metabolites is induced by (methyl) jasmonate, a plant hormone produced in response to stress.

A biosynthesis of many classes of secondary metabolites in plants is induced by the stress hormone, jasmonate. The gene for ORCA-3, a jasmonate responsive APETALA2 (AP2) domain transcription factor was isolated by transferred DNA activation tagging and its over expression resulted in an enhanced expression of several metabolites biosynthetic genes and consequently, an increased accumulation of terpenoid Indole alkaloids. A regulation of metabolites biosynthetic genes by jasmonate responsive AP-2 domain transcription factors may link plant stress responses to changing metabolism. Plants can regulate primary metabolic pathways coordinately with secondary metabolism using a single transcription factor. Since the biosynthesis of many secondary metabolites is induced by jasmonate, the identification of an AP2 domain protein as a regulator of several genes involved in JA responsive metabolism uncovers a control mechanism that may be operative in other stress responsive plant metabolic pathways as well.

Prior Art

US Patent number 5980902 states that the leaves of *Gymnema sylvestre*, a herb belonging to the Asclepiadaecae family have been used by traditional medical practitioners of India to treat diabetic conditions for several centuries. *Gymnema sylvestre* has also been studied for its anti sweet properties, for its ability to inhibit small intestine absorption of glucose and for its ability to suppress increases in blood glucose levels following glucose administration. US Patent number 5900240 refers to *Syzgium cumini* jamun, *Gymnema sylvestre, Momordica charantia* bitter gourd and *Solanum melongena* egg plant, the compositions of which will provide a herbal dietary supplement, which will be tolerated by insulin dependent diabetic sufferers without any undesirable side effects and which will allow blood glucose levels to be controlled to a level below that achievable by administration of insulin. European Patent number WO9842211 has claimed for nutritional supplements, which could be used as a treatment for poor glucose metabolism of Diabetes, and also for prevention of Diabetes by giving the metabolism a boost before the full-blown Diabetes develops. The purpose of Patent number JP4022627 has been to obtain an insulin secretagogue, useful as a curing agent for a patient suffering from hyperglycemia, ketoacidosis etc. instead of insulin.

CN1380072 patent states a medicine for preventing and curing diabetes, other metabolic disease and its complicating diseases. It includes extract of dried or fresh plant *Gymnema sylvestre* and voglepotang sugar, as compared with existent technology it has the advantages of high therapeutic effect, low toxic side effect and long acting time, not only can be used for preventing and curing various diabetes, but also can be used for preventing and curing hyperlipemia, adiposis, arteriosclerosis, X syndrome and other complicating diseases.

US patent 5980902 describes compositions derived from *Gymnema sylvestre* leaf materials that may be administered orally, intravenously, subcutaneously or transdermally which are useful for treating patients having diabetes, impaired glucose tolerance, and various conditions associated with or symptoms of diabetes. Additionally, the compositions reduce polydipsia, polyuria and polyphagia, regenerate the pancreatic islets of Langerhans, including beta cells, increase endogenous insulin, lipase and amylase levels, increase production of proinsulin and c-peptide, and lower blood lipids and triglycerides and free fatty acids.

European patent number WO9510292 talks about glucose metabolism in a human patient being regulated by dosage forms that contain a naturally occurring, plant derived carbohydrate, an aqueous and water-miscible polar solvent extract, preferably an aqueous and ethanolic extract, of *Gymnema sylvestre* in combination with a non-metabolizable polysaccharide preferably a Sterculia urens exudate, in a respective weight ratio in the range of about 1:2 to about 2:1.

Aqueous extracts from the leaves of *G. sylvestre* have been described as inhibiting temporarily the taste of sweet substances. It has also been reported that the raw leaves of *G. sylvestre* have been used in India as a folk medicine for various afflictions including diabetes mellitus. Some fourteen or fifteen different compounds are reported to have been isolated from the leaves of *G. sylvestre* by various techniques. Stocklin, J. Agr. Food Chem. 17(4):704-708 (1969); Sinsheimer, J. Pharm. Sci. 59(5):622-628 (1970). However, applicant is not aware of scientific information as to whether any of the noted chemicals, individually or collectively, contribute to the hypoglycemic properties. It has now been found, however, that the present aqueous and water-miscible polar solvent extract contains at least four fractions that are insulinotropic. Two of these fractions exhibit substantially equal and relatively strong insulinotropic activity.

Chinese patent CN1268515 relates to the extract of *Gymnema sylvestre* which is mainly composed of total triterpene saponin, flavone glycoside, anthocyan,polysaccaride, etc. in which the content of total triterpene saponin is 50-99%, and 25-40% of the total triterpene saponin are six kinds of new triterpene saponin compounds. Said extract possesses the active functions of lowering blood sugar, bloodfat and anti-thrombocyte coagulation.

US patent 6,572,897 describes a composition that contains essential amounts of Alpha Lipoic Acid, Chromium, Lutein, Bioflavonoids(quercetin and rutin), Mormordica Charantia extract, Corosolic Acid, and *Gymnema Sylvestre* Extract, as well as other ingredients and healthy filler ingredients with clinical studies proven to assist in the maintenance of insulin sensitivity and healthy blood sugar levels.

US patent 5,886,029 describes a medicinal composition including a pharmacologically significant quantity of (-)epicatechin augmented with a comparable amount of gymnemic acid useful for the treatment of diabetes in a human subject. The medicinal composition of the invention induces a significant reduction in serum glucose due to the regeneration of pancreatic islet cells. The unique combination of components in the medicinal composition leads to a regeneration of the pancreas cells, which then start producing insulin on their own. Since the composition restores normal pancreatic function, treatment can be discontinued after between about four and twelve months.

US patent 5,137,921 describes the use of an inhibitory agent of an increase in blood sugar level, conduritol A obtained from dried leaves of *Gymnema sylvestre* or from dried bark of Marsdenia condurango by means of extraction.

Shamnugasundaram et al studied the use of *Gymnema Sylvestre* leaf extract in the control of blood glucose in Insulin-dependent Diabetes Mellitus, Journal of Ethnopharmacology 30,pp.281-294,1990.

Baskaran et al studied the antidiabetic effect of a leaf extract from *Gymnema Sylvestre* in Non-insulin-dependent Diabetes Mellitus, Journal of Ethnopharmacology 30, pp. 295-305, 1990. Patients were able to discontinue their conventional drug and maintain their blood glucose homeostasis with the extract of *Gymnema Sylvestre*(GS4) alone. These data suggested that the beta cells might be regenerated/repaired in Type 2 diabetic patients on GS4 supplementation. This is supported by the appearance of raised insulin levels in the serum of patients after GS4 supplementation.

Sugihara Y et al. described the antihyperglycemic effects of gymnemic acid IV, a compound derived from *Gymnema sylvestre* leaves in streptozotocin-diabetic mice, J Asian Nat Prod Res. 2000;2 (4):321-7. Gymnemic acids derived from the methanol extract of leaves of Gymnema sylvestre, at doses of 3.4-13.4mg/kg reduced the blood glucose levels by 13.5-60.0% 6h after the administration, comparable to the potency of glibenclamide. These results indicate that insulin-releasing action of gymnemic acid IV may contribute to the antihyperglycemic effect by the leaves of G. sylvestre. Gymnemic acid IV may be an anti-obese and antihyperglycemic pro-drug.

Rathi et al. studied the effect of *Gymnema sylvestre* on protein-bound polysaccharide components & glycosaminoglycans in experimental diabetes. Indian J. Experimental Biol 19, pp. 715-721, 1981.

Shanmugasundaram et al. tried to find the possible effects of leaf extracts of *Gymnema Sylvestre* on the regeneration of the islets of langerhans in Streptozotocin-diabetic Rats. Journal of Ethnopharmacology 30, pp. 265-279, 1990.

CN1122699 describes capsules made from balsom pear, lagenaria peel, root of Chinese angelica, periwinkle etc. The clinical tests on more than 1000 cases of diabetes patients who do not rely upon insulin therapy verified that the total effective rate is up to 92.5%. It also has notable effect for reducing the blood sugar for diabetes patients relying upon insulin.

Singh SN et al. studied the effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats, J Ethnopharmacol. 2001 Aug;76(3):269-77. Extract at dose 500 mg/kg given orally for 7 and 15 days showed 48.6 and 57.6% hypoglycemic activity, respectively. Prior treatment at the same dose for 30 days provided complete protection against STZ challenge (75 mg/kg/i.p.x1). Results indicate increased metabolization of glucose in treated rats. Increased levels of lipid peroxidation measured as 2-thiobarbituric acid reactive substances (TBARS) indicative of oxidative stress in diabetic rats were also normalized by treatment with the extract.

United States Patent Application 20010021401 relates to an herbal therapeutic product for controlling diabetes mellitus comprising at least one hypoglycemic compound extracted from the pulp of a fruit from a species of genus Eugenia specifically *Eugenia jambolina* and a process for the preparation of the same.

US patent application 20020025349 describes a synergistic oral liquid herbal composition falling under the category of "Asavas" and "Arishtas", useful for management of diabetes, said composition comprising a therapeutically effective amount of plant extracts selected from a. *Momordica charantia* (2-5%), b. *Gymenma sylvestre* (8-12%), c. *Pterocarpus marsupium* (8-12%), d. *Eugenia jambolana* (4-10%), and e. *Trigonella foenum grecum* (1-3%), and, optionally, comprising extracts/powder of *Woodfordia fruticosa* (2 to 5%), *Piper longum* (0.1 to 0.3%), *Elettaria cardamomum* (0.1 to 0.3%), *Myristica fragrans* (0.1 to 0.3%) and *Ammomum subulatum* (0.1 to 0.3%).

US patent 5,972,342 describes mixtures isolated from grains of *Eugenia Jambolana Lamarck*, the preparation of such mixtures, the medicaments containing said mixtures or constituents of said mixtures, and the use of these mixtures for the treatment of diabetes and complications associated with Diabetes.

Kelkar in his article XP-000940531, Phytomedicine Volume 3 (4) pages 353-359,1996/97, described a simple two step purification of antidiabetic compounds from *Eugenia jambolana* fruit-pulp; proteolytic resistance and other properties.

Rathi SS et al., assessed the efficacy of *Momordica charantia* (MC), *Eugenia jambolana* (EJ), *Tinospora cordifolia* (TC) and *Mucuna pruriens* (MP) in the prevention of murine alloxan dibetic cataract. (Phytother Res. 2002 Dec;16(8):774-7). The incidence rate of cataract in MC, EJ, TC and MP treated groups at 120 days was only 0, 0, 1 and 2. Oral feeding of MC, EJ, TC and MP extracts for 1 month produced a fall of 64.33%, 55.62%, 38.01% and 40.17%, respectively, in the serum glucose levels in comparison with the 48 h level. After 2 months of treatment, the respective values were 66.96%, 59.85%, 40.41% and 45.63%. MC and EJ prevented the development of cataract while the protective effect was less with TC and MP along with a significant reduction of plasma glucose levels.

Grover JK et al., studied the amelioration of experimental diabetic neuropathy and gastropathy in rats following oral administration of plant (*Eugenia jambolana, Mucuna pruriens and Tinospora cordifolia*) extracts, Indian J Exp Biol. 2002 Mar;40(3):273-6.

Anila L et al. studied the beneficial effects of flavonoids from *Sesamum indicum, Emblica officinalis* and *Momordica charantia*. Of the three sources, flavonoids isolated from *Emblica officinalis* exerted the

maximum beneficial action by eliciting highly potent hypolipidaemic and hypoglycemic activities. (Phytother Res. 2000 Dec;14(8):592-5)

Vikrant et al. tried to study the efficacy of the extracts of *Momordica charantia* and *Eugenia jambolana* to prevents hyperglycemia and hyperinsulinemia in fructose fed rats, J Ethnopharmacol. 2001 Jul;76(2):139-43.

Sharma SB et al. studied the hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan-induced diabetic rabbits. J Ethnopharmacol. 2003 Apr;85(2-3):201-6.

US patent 6,551,627 15 describes an herbal medicinal composition for preventing or treating type II diabetes. The composition is comprised of extracts from *Pterocarpus marsupium, Morus alba, Orthosiphon aristatus, Opiophogon japonicus, Rosa rugosa, Commelina communis, Trichosanthis kirilowii* and *Anemarrhena asphodeloides*. The patent also describes the effect of the composition in reducing blood glucose level in a patient who has blood glucose level of about 200 to about 300 mg/dl at the beginning of treatment, increasing insulin secretion from pancreatic beta cells and a method of inhibiting degradation of complex carbohydrates to monosaccharides.

US patent 6,448,450 talks about diphenylethylene *Pterocarpus marsupium* which when administered orally decreases blood glucose levels in rats. The compound is an effective anti-diabetic agent that can reduce abnormality of glucose metabolism in diabetes.

European patent application WO0172316 speaks about an edible Ayurvedic herbal composition for reducing blood sugar levels in humans, specially suffering from diabetes mellitus comprising a mixture of ingredients selected from the group consisting of *Cinnamomum zeylanicum, Artocarpus heterophyllus, Salacia reticulata, Tinospora cordifolia* and *Pterocarpus marsupium*. The mixture of the ingredients of the five selected herbs present in therapeutically effective proportions depending on the required strength of the mixture to treat abnormal levels of blood sugar and diabetes mellitus.

US patent 6,562,791 describes a novel glucopyranoside, 6-hydroxy-2-p hydroxybenzylbenzofuran-7-C-.beta.-D-glucopyranoside isolated from *Pterocarpus marsupium* and to a process for the isolation thereof. The invention also relates to a pharmaceutical composition containing 6-hydroxy-2-p-hydroxybenzylbenzofuran-7-C-.beta.-D-glucopyranoside and to method for the treatment of diabetes using said compound.

Manickam M et al. studied the antihyperglycemic activity of phenolics from *Pterocarpus marsupium*, J Nat Prod. 1997 Jun;60(6):609-10. Of the 3 important phenolic constituents of the heartwood of Pterocarpus marsupium, marsupsin (1), pterosupin (2), and pterostilbene (3), Marsupsin and pterostilbene significantly lowered the blood glucose level of hyperglycemic rats, and the effect was comparable to that of 1,1-dimethylbiguanide (metformin).

Sheehan EW discovered a constituent of *Pterocarpus marsupium*, (-)-epicatechin, as a potential antidiabetic agent. J Nat Prod. 1983 Mar-Apr;46(2):232-4. (-)-Epicatechin was found to reverse hyperglycemia in alloxan diabetic rats when given before or within 24 hr after the dose of alloxan.

However, when doses of (-)-epicatechin (30 mg/kg, i.p., twice daily for 3 days) are begun 92 hr after alloxan, there is no significant difference in blood glucose levels between control and (-)-epicatechin treated rats.

Gupta SS et al. studied the effect of *Tinospora cardifolia* on fasting blood sugar level, glucose tolerance and adrenaline induced hyperglycaemia. Indian J Med Res. 1967Jul;55(7):733-45.

Prince PS et al. studied the antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. J Ethnopharmacol. 1999 Jun;65(3):277-81. *T. cordifolia* root extract (TCREt) (2.5 and 5.0 g/kg) for 6 weeks resulted in a decrease in the levels of plasma thiobarbituric acid reactive substances, ceruloplasmin and alpha-tocopherol in alloxan diabetic rats. The root extract also causes an increase in the levels of glutathione and vitamin C in alloxan diabetes. The root extract at a dose of 5.0 g/kg showed the highest effect. The effect of TCREt was more effective than glibenclamide.

Stanely P et al. studied the hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats, J Ethnopharmacol. 2000 Apr;70(1):9-15. Oral administration of an aqueous T. cordifolia root extract (TCREt) to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids. The extract caused an increase in body weight, total haemoglobin and hepatic hexokinase. The root extract also lowers hepatic glucose-6-phosphatase and serum acid phosphatase, alkaline phosphatase, and lactate dehydrogenase in diabetic rats.

Li M et al. studied the hypoglycemic effect of saponin from *Tribulus terrestris*, Zhong Yao Cai. 2002 Jun; 25(6): 420-2. The level of serum glucose could be significantly reduced by saponin from *Tribulus terrestris*, which was the rate of 26.25% and 40.67% in normal mice and diabetic mice in respectively. The level of serum triglyceride could be reduced 23.35%.

US patent 6,042,834 describes a herbal composition for the treatment of diabetes, comprising 15 percent by weight of dried, powdered seeds of *Trigonella foenum-graecum*; 23 percent by weight of dried, powdered seeds of *Nigella sativa*; 10 percent by weight of dried, powdered leaves of *Origanum vulgare*; 10 percent by weight of dried, powdered sap of *Rosmarinus officinalis*; 15 percent by weight of dried, powdered beans of *Lupinus termis*; 12 percent by weight of dried, powdered black leaves of Lawsonia inermis; and 15 percent by weight of dried, powdered seeds of *Foeniculum vulgare*.

An Indian patent application 305/MAS/99 describes a process for preparation of an antidiabetic herbal drug from the plants *trichopus zeylanicus, withania somnifera* and *piper longum*.

Andallu B et al studied the hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera*, Dunal) root, Indian J Exp Biol. 2000 Jun;38(6):607-9. Hypoglycemic, diuretic and hypocholesterolemic effects of roots of *W. somnifera* (ashvagandha) were assessed on human subjects. Decrease in blood glucose was comparable to that of an oral hypoglycemic drug. Significant increase in urine sodium, urine volume, significant decrease in serum cholesterol, triglycerides, LDL (low density lipoproteins) and VLDL (very low density lipoproteins) cholesterol were observed indicating that root of *W. somnifera* is a potential source of hypoglycemic, diuretic and hypocholesterolemic agents.

US patent 5,470,879 describes a process for stimulating the secretion of insulin and for the treatment of non-insulin dependent diabetes by the administration of effective quantities of substantially pure 4-hydroxyisoleucine or its lactone form or mixtures thereof obtained from *Trigonella foenum graecum L.*

Vats V et al. evaluated the anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats, J Ethnopharmacol. 2002 Jan;79(1):95-100.

Abdel-Barry JA et al. studied the hypoglycemic effect of aqueous extract of the leaves of *Trigonella foenum-graecum* in healthy volunteers, East Mediterr Health J. 2000 Jan;6(1):83-8.

Ali L et al. characterized the hypoglycemic effects of *Trigonella foenum graecum* seed, Planta Med. 1995 Aug; 61(4):358-60. The whole powder of *Trigonella foenum graecum* seeds and its extracts were tested for their hypoglycemic effect on normal and diabetic model rats. The powder, its methanol extract, and the residue remaining after methanol extraction had significant hypoglycemic effects when fed simultaneously with glucose.

Zia T et al. evaluated the oral hypoglycaemic effect of *Trigonella foenum-graecum* L. (methi) in normal mice, J Ethnopharmacol. 2001 May;75(2-3):191-5. The presence of hypoglycemic activity in aqueous and methanolic extract indicates that the active compounds are polar in nature.

Gupta A et al. studied the effect of *Trigonella foenum graecum* (fenugreek) seeds in glycemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. J Assoc Physicians India. 2001 Nov; 49:1057-61. Adjunct use of fenugreek seeds improves glycemic control and decreases insulin resistance in mild type-2 diabetic patients. There is also a favorable effect on hypertriglyceridemia.

Sharma RD et al. studied the effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes, Eur J Clin Nutr. 1990 Apr;44(4):301-6. The fenugreek diet significantly reduced fasting blood sugar and improved the glucose tolerance test. There was a 54 per cent reduction in 24-h urinary glucose excretion. Serum total cholesterol, LDL and VLDL cholesterol and triglycerides were also significantly reduced. The HDL cholesterol fraction, however, remained unchanged. These results indicate the usefulness of fenugreek seeds in the management of diabetes.

In US Patent number 6391854, a water-soluble fraction of *Momordica charantia* and methods for its preparation and use in the treatment of hyperglycaemic disorders, is provided for. In Patent number RO116044 patent application, the process of extracting the immature, dried, milled fruit of *Momordica charantia* with methanol and concentrating it in an ethanolic solution till a viscous product is obtained, is a process for preparing a medicine for internal use and used as an adjuvant in the therapy of Diabetes *mellitus*. In US Patent number 6127338, a water-soluble extract of *Momordica charantia*, methods for its preparation and use in the treatment of hyperglycaemic disorders are provided and so also in Patent number WO9843484.

In US Patent number 6043824, a herbal composition for the treatment of Diabetes comprising therein of *Trigonella foenum-graecum, Nigella sativa, Origanum vulgare, Rosmarinus officinalis, Lupinus termis, Lawsonia inermis* and *Foeniculum vulgare* in a capsule form has been claimed. US Patent number 5886029 alludes to a medicinal invention, which results in a significant reduction in serum glucose due to the regeneration of pancreatic islet cells. The medicinal composition includes Gymnemic acid, *Cinnamomum tamala, Syzgium cumini, Trigonella foenum graceum, Azardichta indica, Ficus racemosa* and *Tinospora cordifolia*. This combination leads to a regeneration of pancreas cells, which then start producing insulin on their own.

Patent number WO0172316 describes an edible ayurvedic herbal composition for reducing the blood sugar level in humans suffering from Diabetes *mellitus* comprising therein, a mixture of ingredients selected from *Cinnamomum zeylanicum, Artocarpus heterophyllus, Salacia reticulata, Tinospora cordifolia* and *Pterocarpus marsupium* in therapeutically effect proportions to treat abnormal levels of blood sugar and Diabetes *mellitus*.

US Patent number 5917052 states that no prior study has described any hypoglycaemic activity or extracts of *Cryptolepis* sp or that quindoline alkaloids such as cryptolepine or quindoline would be useful as hypoglycaemic agents. The invention provides a method for the use of extracts from *Cryptolepis* sp and compounds of the quindoline family of alkaloids such as quindoline, cryptolepine etc. as well as pharmaceutically acceptable salts thereof as hypoglycaemic agents or as agents to lower triglyceride levels, particularly in diabetic subjects.

In US Patent number 5837255, it has been held that no prior study has described any hypoglyemic activity of extracts of *Harungana* spp or *Vismia* spp nor was there any prior suggestion that anthracenone compounds such as harunganin or vismin are useful as hypoglycaemic agents. The invention provides a method for the use of extracts from *Harunganin* spp or from *Vismia* spp and for the use of anthracenone compounds harunganin and vismin, as well as pharmaceutically acceptable salts thereof as hypoglycaemic agents or agents to lower blood glucose levels, particularly in diabetic subjects.

US Patent number 2002041904 has mentioned that in recent years among therapeutic drugs classified as anti diabetic agents, alpha glucosidase inhibitors which inhibit the activity of alpha-glucosidase have been widely used in the treatment of Diabetes and pre-Diabetes. *Salacia reticulata* has been used since ancient times in the ancient medicines of India and Sri Lanka. The object of this invention has been to provide a novel compound which is extracted from the woody climbing plants, *Salacia prinoides* and *Salacia oblonga* and is superior in terms of its characteristic of inhibiting the activity of alpha-glucosidase (compound being referred to as 'alpha-glucosidase inhibitor'). In US Patent number 5691386, it has been reported that plant of the genus *Salacia* has been used to treat Diabetes. In India, a hot water extraction of the whole plant *Salacia prinoides* has been taken orally as an anti-diabetic (P.N. Mehra et al., Res. Bull Punjab University Sci 20: 487-502 (1969). In Sri Lanka aqueous extracts of the roots of *Salacia reticulata* have been used in the treatment of Diabetes *mellitus* (E.H. Karunanayake et al., J. Ethnopharmacol 13 (2): 227-228 (1985). This invention claims to provide a novel triterpenoid compound, 3-beta,30–dihydroxylup-20-29-en-2 one as well as pharmaceutically acceptable salts thereof, having hypoglycaemic activity, hypoglycemic compositions comprising the novel triterpenoid

compound in purified form as well as methods for their use, as an hypoglycaemic agent. The invention further encompasses compositions comprising the triterpenoid compounds in purified form or pharmaceutically acceptable salts for use as hypoglycaemic agent, useful for the treatment of Diabetes.

US Patent number 5916567 relates to a herbal therapeutic product for treating Diabetes and relates to a therapeutic product processed from the seed of a plant from the family Leguminosea, whose fibres affect the blood sugar level by increasing the viscosity of the unstirred layer between food and the lining of the intestines and the stomach thereby making the carbohydrates available for absorption at a slower rate.

US Patent number 5,470,873 discloses a composition for the treatment of NIDDM comprising therein, maltol, obtained from Ginseng roots and an extract obtained from Orthosiphon aristatus, effective in regulating blood glucose levels in diabetic animals but this by itself is not sufficient to normalise the glucose levels, nor was any lasting effect determined following termination of the treatment.

The present invention relates to an amalgam of 15 (Fifteen) different traditional Indian medicinal plants, the compounds of which have been identified and isolated in order to have the same screened to ascertain their therapeutic effects in treating Diabetes, more specifically type II Diabetes with the compositions derived from non-toxic medicinal plants and effective in the prevention, treatment and cure of NIDDM.

Brief description of the tables and figures:

Table 1 gives the list of medicinal plants used to arrive at the formulation, AGT_D_For_0001.

Table 2 gives the list of extracts from individual plants.

Table 3 shows a representative mass spectral peak grid arrived at using a comparative mass spectral peak analysis approach that illustrates the components that are either common or unique to individual extracts.

Table 4 shows the comparative results of the mass spectrometry analysis of the chloroform-methanol fractions of formulation AGT_D_For_0001_05:03 and its constituents, AGT_D_Mch_Se_05:03, AGT_D_Tfg_Se_05:03, AGT_D_Ej_Se_05:03 & AGT_D_Gs_Ml_05:03 indicating the common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z)

Table 5 shows the comparative results of the mass spectrometry analysis of the methanolic fractions of formulation AGT_D_For_0001_05 and its constituent plants, AGT_D_Mch_Se_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05 and AGT_D_Gs_Ml_05.

Table 6 fives an estimate of total number of constituents contained in a few of the individual plant extracts that have been characterized using HPLC-based metabolite fingerprinting.

Table 7 summarizes the comparative HPLC-based fingerprinting analysis done in the case of different plant extracts using a few representative instances (Ma_Le_Wa_01 v/s Ws_Ro_Wa_01; Ca_Ro_Et_01 v/s Ws_Ro_Et_01 and Ej_Se_Me_01 v/s Tt_Fr_Me_01).

Figure 1 (A-F) gives the representative mass spectra of methanolic extracts (AGT_D_Gs_Ml_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05) and chloroform extracts (AGT_D_Gs_ML_03, AGT_D_Tfg_Se_03, and AGT_D_Ej_Se_03)

Figure 2 (A-E) gives the representative mass spectra of the comparative mass spectrometric analysis between the chloroform fractions of the formulation, AGT_D_For_0001_03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_03, AGT_D_Ej_Se_03, AGT_D_Tfg_Se_03 and AGT_D_Gs_Ml_03.

Figure 3 (A-E) gives the representative mass spectra of the comparative mass spectrometric analysis between the chloroform –methanol fractions of the formulation, AGT_D_For_0001_05:03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_)5:03, AGT_D_Ej_Se_)5:03, AGT_D_Tfg_Se_05:03 and AGT_D_Gs_Ml_05:03.

Figure 4 (A-E) gives the representative mass spectra of the comparative mass spectrometric analysis between the methanol fractions of the formulation, AGT_D_For_0001_05 and the methanol extracts of a few of its constituents, AGT_D_Mch_Se_05, AGT_D_Ej_Se_05, AGT_D_Tfg_Se_05 and AGT_D_Gs_Ml_05.

Figure 5 gives the chromatograms of extracts Ma_Le_Wa_01 and Ws_Ro_Wa_01 at selected wavelengths

Figure 6 gives the chromatograms of extracts Cr_Ro_Et_01 and Ws_Ro_Et_01 at selected wavelengths

Figure 7 gives the chromatograms of extracts Ej_Se_Me_01 and Tt_Fr_Me_01 at selected wavelengths

Description

The object of the invention is to provide edible herbal dietary supplements. The invention relates to a method and a composition for potentiating insulin activity to treat Diabetes patients. This composition has an effect on smoothing out fluctuations in the glucose levels.

The insulin potentiating agents are natural substances derived from plant extracts and can be safely consumed by humans. This naturally derived agent has an advantage in that it does not cause side effects. These agents can be used with conventional drug treatments like oral hypoglycemic agent or insulin.

One of the main problems that have been reported in the case of phyto-formulations is the lack of clarity in terms of comprehensive qualitative and quantitative characterization of all the detectable components present in the mixture. The availability of such information about phyto-extracts will play a major role in the scientific validation and standardization of both the therapeutic effects and constituents present in these phytoextracts.

Metabolite profiling has emerged as a robust tool that is fast, reliable, sensitive and suitable for automation, covering a significant number of metabolites. A range of analytical technologies enhances the sensitivity and universality of mass spectrometry by chromatographic separations. Although the use of multi- target profiling had been earlier limited to rapid clinical detection of human diseases, metabolic screening approaches using mass spectrometry are being increasingly used in plant research at present. A major advantage of mass-spectrometry is that unknown peaks can be determined as reliably as known target analytes without prior knowledge of their exact chemical structure. Studies using gas chromatography/mass spectrometry (GC/MS) have automatically quantified 326 distinct compounds from *Arabidopsis thaliana* leaf extracts. It has been possible to assign a chemical structure to approximately half of these compounds. Comparison of four *Arabidopsis* genotypes (two homozygous ecotypes and a mutant of each ecotype) showed that each genotype possesses a distinct metabolic profile. Data mining tools such as principal component analysis enabled the assignment of "metabolic phenotypes" using these large data sets. The results of this study have shown that metabolic phenotypes of the two ecotypes were more divergent than were the metabolic phenotypes of the single-loci mutant and their parental ecotypes. These results demonstrate the use of metabolite profiling as a tool to significantly extend and enhance the power of existing functional genomics approaches. Due to the increased chemical complexity and diversity at the metabolite level in higher plants, no singular technique exists for profiling all cellular metabolites concurrently. This problem can be approached through the division of metabolites into major profiling classes, *i.e.* triterpenoids, phenolics, lipids, carbohydrates, amino acids and carbohydrates. The hyphenated mass spectrometric techniques such as GC/LC/ESI- MS provide both relative quantitative abundances and specific information that can be utilized in chemical identification. Methods have been developed using HPLC interfaced with an ion trap mass spectrometer capable of sequential tandem mass spectrometry for profiling plant metabolites, *i.e.* HPLC-ESI-MS^n. This approach has been used to profile saponin glycosides in multiple cultivars of alfalfa followed by the comparison of these profiles to the model legume *M. truncatula*. To date, twenty-seven novel saponin glycosides in *M. truncatula* have been identified using this technology. This technology was also used to identify novel malonated saponin glycosides in alfalfa and *M. truncatula*.

Metabolite grid (*Metagrid*)

The plants selected for the isolation of therapeutically relevant extracts/molecules ("Yezdex") to be used in the treatment of Diabetes, are being subjected to both targeted and non-targeted screening procedures. The ongoing-targeted screening procedures, which feature a comprehensive metabolite profiling of multitudes of phyto-extracts, were envisaged to facilitate the creation of a metabolite grid. Extensive comparative analyses of the individual plant species with the existing drug and/or phytoextract formulations in the market has revealed the presence of both unique and common molecular constituents that can be used individually and/or in combination to accelerate the process of discovery of novel therapeutic formulation. (Figure 8)

Screening Methodologies:

Extraction:

Method 1: The successive extraction from various medicinal plants parts was carried out using soxhlet extractor. The solvents used, were based on their sequential polarity starting from non-polar to polar, wherein, various classes of metabolites were extracted viz; petroleum ether (phytosterols, fixed oils and fats), benzene (fixed oils and fats), chloroform (alkaloids), acetone (phytosterols, phenolics and tannins) ethanol (alkaloids, carbohydrates, glycosides, phytosterols, saponins, phenolics, tannins, proteins and amino acids) and water (alkaloids, carbohydrates, glycosides, saponinns, phenolics, tannins, proteins, amino acids, gums and mucilage) at 65°C. These fractions were lyophilized and stored in amber colored bottles at 4°C. (Figure 9 & 10)

Phytochemical investigations were also carried out on these extracts using various tests like Mayer's and Dagendorf's tests for alkaloids; Molisch, Fehling and Benedict tests for carbohydrates; Lieberman Buchard's test for phytosterols and triterpenes; spot test for fixed oils and fats; Ferric chloride and Lead acetate test for phenolic compounds and tannins; Ninhydrin and Biuret tests for protein and aminoacids; alcoholic precipitation followed by Molisch test for gum and mucilages.

Method 2: To characterize a particular class of metabolites, fractional extraction procedure was adopted. In this method, various metabolite classes were screened like polysaccharides, terpenoids, phenolics, alkaloids, oils, fat and waxes present in the medicinal plants parts. (Figure 11)

High Performance Liquid Chromatography ("HPLC") profiling:

The extracted fractions were subjected to HPLC using μ bondapak C_{18} column (Waters Alliance 2695 Separation Module) to separate the constituent metabolites. The fractions were eluted using a combination (80:20, 60:40, 50:50, 40:60, 20:80) of methanol:water / acetonitrile:water. The gradient run was also carried out wherever required. 5- 10ul of sample was injected with flow rate of 1ml/min and HPLC run was performed for 30 minutes. The detection was carried out on photodiode array and the analysis of the results was done with the help of Millennium™ software.

Identification and characterization of purified/partially purified extracts by MALDI TOF:

The metabolites were identified and characterized by using the MALDI –TOF Voyager system 4266. The matrix for MALDI-TOF used was alpha cyano-4-hydroxycinnamic acid. Nuclear Magnetic Resonance (NMR) will be performed for unique and common fallouts of *Metagrid* for it structure elucidation.

Comprehensive constituent profiling and creation of the Metagrid:

A comparative profile of a therapeutic formulation and its individual constituents has been worked upon. AGT_D_For_0001 (See Table 1) , a therapeutic formulation, which comprises of approximately 15 (Fifteen) medicinal plants and their comparative analyses has been undertaken using mass spectrometry (MALDI-TOF MS). In addition to the formulation, 51 (Fifty One) individual plant extracts (see Table 2) have been comprehensively profiled using HPLC. Representative analytical data of the biochemical profiling carried out thus far is shown below:

Mass-spectrometry based comprehensive constituent profiling of the formulation AGT_D_For_0001 and the constituent plants

Example1: The methanolic and ethanolic extracts of Tfg, Ej and Gs were analysed using the screening method 1 as described earlier. The methanolic extracts AGT_D_Gs_Ml_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05, and chloroform extracts AGT_D_Gs_ML_03, AGT_D_Tfg_Se_03, AGT_D_Ej_Se_03 when compared, revealed common mass spectral peaks in both the solvents, viz. m/z 104, 112, 176, 184, 212, 228, 241, 496, 522, 592, 606. (As shown in Table 3 and Figure 1) It was also observed that molecular mass spectral peaks m/z 203, 267, 337, 634, 694 were unique to the methanolic extracts and molecular mass spectral peaks m/z 190, 336, 340, 390, 520, 621 were unique to the chloroform extracts.

Example2: A comparative profiling of the therapeutic formulation, AGT_D_For_0001, and its individual constituents, mentioned supra, was carried out to isolate terpenoids/ phenolics, using method 2 as previously described. The comparative results of the mass spectrometry analysis between the chloroform fractions of the formulation, AGT_D_For_0001_03 and its constituents, AGT_D_Mch_Se_03, AGT_D_Ej_Se_03, AGT_D_Tfg_Se_03, AGT_D_Gs_Ml_03 revealed a few common mass spectral peaks m/z 104, 138, 172, 184, 336, indicating the presence of terpenoids/ phenolics which may play a significant role in the treatment of diseases. The mass spectral peaks such as m/z- 212, 288, 338, 496.3, 520.39, 623.48 that are present in formulation but are also uniquely present in few of the medicinal plant parts analysed thus far indicates, that this system can be utilized to track the origin of these specific compounds to specific plants or plant parts used to arrive at a therapeutic formulation. (As shown in Figure 2)

Example 3: The basic alkaloids were extracted by method 2 and were profiled by HPLC and mass spectrometry. The comparative results of the mass spectrometry analysis the chloroform-methanol fractions of formulation AGT_D_For_0001_05:03 and its constituents, AGT_D_Mch_Se_05:03, AGT_D_Tfg_Se_05:03, AGT_D_Ej_Se_05:03, AGT_D_Gs_Ml_05:03 reveals the common mass, m/z 104, 138, 172, 184, 336 and is suggestive of presence basic alkaloids and its precursors, which may have significant role in the treatment of diseases. As mentioned in example 2, the mass spectral peaks such as m/z- m/z 112, 155, 212, 286, 288, 338, 352.16, 496.38, 520.39, 623.48 that are present in formulation but are also uniquely present in few of the medicinal plant parts analyzed thus far indicates, that this system can be utilized to track the origin of these specific compounds to specific plants or plant parts used to arrive at a therapeutic formulation. (As shown in Table 4 & Figure 3)

Example 4: The quaternary alkaloids were extracted by method 2 and were profiled by HPLC and mass spectrometry. The comparative results of the mass spectrometry analysis of the methanolic fractions of formulation AGT_D_For_0001_05 and its constituent plants, AGT_D_Mch_Se_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05 and AGT_D_Gs_Ml_05 revealed the presence of common mass spectral peaks m/z 104, 172, 190, 212, 228, 294, 379 that are representative of quaternary alkaloids, N-oxides and their precursors. As mentioned in example 2 and 3, the mass spectral peaks such as m/z- 118.1, 138.06, 241.17, 250.1, 265.97, 296, 345, 441.05, 443.04, 492.98 that are present in formulation but are also uniquely present in few of the medicinal plant parts analyzed thus far indicates, that this system can be utilized to track the origin of these specific compounds to specific plants or plant parts used to arrive at a therapeutic formulation. (As shown in Table 5 & Figure 4)

HPLC based comprehensive constituent profiling and metabolite fingerprinting of individual plant extracts:

The extracted fractions were subjected to HPLC using μ bondapak C_{18} column (Waters Alliance 2695 Separation Module) to separate the constituent metabolites. The fractions were eluted using a combination (80:20, 60:40, 50:50, 40:60, 20:80) of methanol:water / acetonitrile:water. The gradient run was also carried out wherever required. 5- 10ul of sample was injected with flow rate of 1ml/min and HPLC run was performed for 30 minutes. The detection was carried out on photodiode array and the analysis of the results was done with the help of Millennium™ software.

Comprehensive constituent profiling and metabolite fingerprinting of individual 51 individual plant extracts (see table 2) has been carried out. (See Table 6 and Figure 5)

Comparative metabolite fingerprinting analysis has revealed the presence of both common and unique constituents that are present in individual plant extracts that have been extracted under similar conditions. (See Table 7)

The intuitive methodology used to arrive at the data (using a combinatorial matching of retention times and absorption maxima) summarized in table 7 is illustrated using a few representative instances. shown in Figure 6 & 7.

The extracts were randomly screened for metabolites by HPLC and mass spectrometry, the results reveal the group of common metabolites in most of the extracts and some unique molecular masses were also observed which would be further subjected to structural elucidation and characterization by MS^n and NMR. Furthermore, derivatisation of these characterized molecules will be carried out using the synthetic chemistry approach.

Carbon dioxide extraction procedure would be carried as against the conventional method of extraction, expecting more efficient extraction with less solvent consumption and shorter extraction time.

Screening of Extracts for its bioactivity:

51 (Fifty One) individual extracts and their combinations are ready for primary and secondary assay systems for Diabetes.

The extracts will be tested for primary assay:

a. using murine pancreatic islet cell lines (***HIT, HIP, RIN, alpha-TC and beta-TC***) to monitor the changes in levels of insulin-secretion in response to the treatment with defined phyto-extracts
b. changes in insulin-resistance will also be monitored in the murine adipocyte cell line- ***3T3-L1*** in response to the treatment with defined phytoextracts.

Secondary bioassay using mouse models will be conducted to validate the phytoextracts. Different kinds of mouse models will be used for this purpose

a. streptozotocin (STZ) /alloxan induce diabetic mouse.
b. Genetically modified mouse models (db/db,db/ob,and ob/ob)

The gene for ORCA-3, a jasmonate responsive APETALA2 (AP2) domain transcription factor may be isolated by transferred DNA activation tagging and expressed in these therapeutic plants expecting an enhanced expression of several metabolites biosynthetic genes and consequently, an increased accumulation of secondary metabolites of interest in the treatment of diabetes and allied disorders.

Table 1: List of medicinal plants used to arrive at the formulation, AGT_D_For_0001

Formulation AGT_D_For_0001		Botanical name
1	Amlaki	*Phyllanthus emblica*
2	Guduchi	*Tinospora cordifolia*
3	Nimbha	*Azadiractha indica*
4	Jambu	*Eugenia jambulana*
5	Medhika	*Trigonella foenum graceum*
7	Haritaki	*Terminalia chebula*
8	Vibhitaki	*Mucunapuriens*
9	Haridra	*Curcuma longa*
10	Udumbara	*Ficus glomerata*
11	Bhumyamalaki	*Phyllanthus niruri*
12	Ashwagandha	*Withania somenifera*
13	Karavalli	*Momordica charantia*
14	Meshasringi	*Gymnema sylvestre*
15	Silajit	*Euphorbia royleana*

Table 2: List of extracts from individual plants

Extract ID	*Plant name*	*Tissue*	*Solvent*
Cr_Ro_Me_01	*Catharanthus roseus*	Root	methanol
Cr_Ro_Et_01	*Catharanthus roseus*	Root	ethanol
Cr_Ro_Ch_01	*Catharanthus roseus*	Root	chloroform
Ej_Se_Me_01	*Eugenia jambolana*	Seed	methanol
Ej_Se_Et_01	*Eugenia jambolana*	Seed	ethanol
Ej_Se_Ch_01	*Eugenia jambolana*	Seed	chloroform
Ej_Se_Pe_01	*Eugenia jambolana*	Seed	petroleum ether
Ej_Se_Be_01	*Eugenia jambolana*	Seed	benzene
Ej_Se_Et_01(20)	*Eugenia jambolana*	Seed	ethanol-20%
Ej_Se_Wa_01	*Eugenia jambolana*	Seed	water
Eo_Fr_Me_01	*Emblica officinalis*	Fruit	methanol
Eo_Fr_Ch_01	*Emblica officinalis*	Fruit	chloroform
Gs_Le_Me_01	*Gymnema sylvestre*	Leaf	methanol
Gs_Le_Et_01	*Gymnema sylvestre*	Leaf	ethanol
Gs_Le_Ch_01	*Gymnema sylvestre*	Leaf	chloroform
Gs_Le_Pe_01	*Gymnema sylvestre*	Leaf	petroleum ether
Gs_Le_Be_01	*Gymnema sylvestre*	Leaf	benzene
Gs_Le_Et_01(20)	*Gymnema sylvestre*	Leaf	ethanol-20%
Gs_Le_Wa_01	*Gymnema sylvestre*	Leaf	water
Ma_Le_Me_01	*Melia azadirechta*	Leaf	methanol
Ma_Le_Et_01	*Melia azadirechta*	Leaf	ethanol
Ma_Le_Ch_01	*Melia azadirechta*	Leaf	chloroform
Ma_Le_Pe_01	*Melia azadirechta*	Leaf	petroleum ether
Ma_Le_Be_01	*Melia azadirechta*	Leaf	benzene
Ma_Le_Hx_01	*Melia azadirechta*	Leaf	hexane
Mc_Fr_Me_01	*Morinda citrifolia*	Fruit	methanol
Mc_Fr_Et_01	*Morinda citrifolia*	Fruit	ethanol
Mc_Fr_Ch_01	*Morinda citrifolia*	Fruit	chloroform
Pm_Ba_Et-01	*Pterocarpus marsupium*	Bark	ethanol
Pm_Ba_Wa-01	*Pterocarpus marsupium*	Bark	water
Tc_Fr_Me_01	*Tinospora cardifolia*	Fruit	methanol
Tc_Fr_Et_01	*Tinospora cardifolia*	Fruit	ethanol
Tc_Fr_Ch_01	*Tinospora cardifolia*	Fruit	chloroform
Tt_Fr_Me_01	*Tribulus teristris*	Fruit	methanol
Tt_Fr_Et_01	*Tribulus teristris*	Fruit	ethanol
Tt_Fr_Ch_01	*Tribulus teristris*	Fruit	chloroform
Tt_Fr_Pe_01	*Tribulus teristris*	Fruit	petroleum ether

Tt_Fr_Be_01	*Tribulus teristris*	Fruit	benzene
Tfg_Se_Me_01	*Trigonella foenum graecum*	Seed	methanol
Tfg_Se_Et_01	*Trigonella foenum graecum*	Seed	ethanol
Tfg_Se_Ch_01	*Trigonella foenum graecum*	Seed	chloroform
Tfg_Se_Pe_01	*Trigonella foenum graecum*	Seed	petroleum ether
Tfg_Se_Be_01	*Trigonella foenum graecum*	Seed	benzene
Tfg_Se_Et_01(20)	*Trigonella foenum graecum*	Seed	ethanol-20%
Tfg_Se_Wa_01	*Trigonella foenum graecum*	Seed	water
Ws_Ro_Me_01	*Withania somnifera*	Root	methanol
Ws_Ro_Et_01	*Withania somnifera*	Root	ethanol
Ws_Ro_Ch_01	*Withania somnifera*	Root	chloroform
Ws_Ro_Pe_01	*Withania somnifera*	Root	petroleum ether
Ws_Ro_Be_01	*Withania somnifera*	Root	benzene
Ws_Ro_Wa_01	*Withania somnifera*	Root	Water

Table 3 shows a representative mass spectral peak grid arrived at using a comparative mass spectral peak analysis approach that illustrates the components that are either common or unique to individual extracts.

Molecular Masses of constituents (m/z)common to both methanolic and ethanolic extracts	Molecular Masses of components (m/z), unique to Methanolic extracts	Molecular Masses of components (m/z), unique to chloroform extracts
104	203	190
112	267	336
176	337	349
184	534	390
212	594	520
228		621
241		
496		
522		
558		
592		
606		

Table 4 Comparative results of the mass spectrometry analysis of the chloroform-methanol fractions of formulation AGT_D_For_0001_05:03 and its constituents, AGT_D_Mch_Se_05:03, AGT_D_Tfg_Se_05:03, AGT_D_Ej_Se_05:03 & AGT_D_Gs_Ml_05:03 indicating the common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z)

Common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z)	Mass spectral peaks that are uniquely present in the constituent plants (m/z)	
104	112	Ej
138	155	Gs
172	212	Gs, Ej
184	286	Ej
336	288	Tfg
	338	Ej
	352.16	Mch, Ej
	496.38	Mch, Tfg
	520.39	Mch, Tfg
	623.48	Mch, Ej, Gs

Table 5 The comparative results of the mass spectrometry analysis of the methanolic fractions of formulation AGT_D_For_0001_05 and its constituent plants, AGT_D_Mch_Se_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05 and AGT_D_Gs_Ml_05.

Common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z))	Mass spectral peaks that are uniquely present in the constituent plants (m/z)	
104.1	118.1	Tfg
	138.06	Mch, Tfg
172.05	241.17	Mch, Tfg
190.07	250.1	Ej
207.1	265.97	Ej, Mch, Gs
212.05	296	Gs
228.01	342	Ej
294.1	441.05	Ej, Tfg, Gs
379.1	443.04	Ej, Gs
	492.98	Gs

Table 6: Estimation of total number of constituents contained in a few of the individual plant extracts that have been characterized using HPLC-based metabolite fingerprinting.

Extract ID		Constituents in		Total number of constituents
		UV Range	Visible Range	
1	Eo_Fr_Me_01	123	603	726
2	Cr_Ro_Me_01	232	674	906
3	Ws_Ro_Me_01	247	590	837
4	Tfg_Se_Me_01	176	604	780
5	Ej_Se_Me_01	150	656	806
6	Tt_Fr_Me_01	193	507	700
7	Mc_Fr_Me_01	169	615	784
8	Tc_Fr_Me_01	226	561	787
9	Ws_Ro_Et_01	218	598	816
10	Tfg_Se_Et_01	152	616	768
11	Ws_Ro_Wa_01	159	618	777
12	Ma_Le_Et_01	221	540	761
13	Cr_Ro_Et_01	229	533	762
14	Ej_Se_Et_01	109	600	709
15	Gs_Le_Et_01	191	552	743
16	Gs_Le_Me_01	237	557	794
17	Ma_Le_Wa_01	114	643	757

Table 7 summarizes the comparative HPLC-based fingerprinting analysis done in the case of different plant extracts using a few representative instances (Ma_Le_Wa_01 v/s Ws_Ro_Wa_01; Ca_Ro_Et_01 v/s Ws_Ro_Et_01 and Ej_Se_Me_01 v/s Tt_Fr_Me_01).

Extract ID		Total No. of constituents	Common Constituents	Unique constituents
1	Ma_Le_Wa_01 Ws_Ro_Wa_01	757 777	7	750 770
2	Ca_Ro_Et_01 Ws_Ro_Et_01	762 816	41	721 775
3	Ej_Se_Me_01 Tt_Fr_Me_01	806 700	40	766 660

CLAIMS

1. A screening method of arriving at a formulation, comprising of a combination of various herbal extracts from potent Indian medicinal plants, effective in the treatment and cure of Diabetes and its associated problems like cardiac diseases, renal failure, eye diseases, neuropathy, obesity and a host of other diseases.
2. A claim as in claim 1 wherein the metabolites present in the formulation can be used individually as a drug molecule.
3. A claim as in claim 2 wherein the whole combination of metabolites present in the formulation can be used a drug molecule.
4. A claim as in claim 3 wherein the formulation of a drug molecule can be arrived at by different metabolite class combinations.
5. A claim as in claim 1 wherein each individual herbal extract exhibiting properties can play a significant role in the treatment and cure of diabetes and its associated problems.
6. A claim as in claim 5 wherein the extracts can be used in various combinations to play an effective role to provide a synergistic effect in the treatment of diabetes and related risks.
7. A claim as in claim 5 wherein the metabolites present in each individual extract can be used as a therapeutic agent to treat diabetes and allied disorders.
8. A claim as in claim 5 wherein the combination of various metabolites from the different extracts can be used as a drug molecule in treating diabetes and associated risks.
9. A claim as in claim 1 wherein the screening method adopted is effective in the isolation, identification and characterization of molecules in isolation.
10. A claim as in claim 1 wherein the screening method adopted can play a useful role in the isolation, identification and characterization of a therapeutic class of molecules.
11. A claim as in claim 9 wherein the molecule identified can be applied for derivatisation of analogues.
12. A claim as in claim 11 wherein the derivatized molecule renders greater efficacy in treating Diabetes and allied disorders.
13. A claim as in claim 1 wherein the formulation can be administered sequentially and/or simultaneously.
14. A claim as in claim 13 wherein the formulation is rendered suitable for oral and peritoneal including subcutaneous, intramuscular, intravenous and intradermal administration.
15. A claim as in claim 14 wherein the dosage can be administered in the form of tablets, pills, powders, solutions, syrups, suspensions, emulsions, granules, capsules and suppositories.

ABSTRACT

The present invention relates to a unique method, *Metagrid,* for selecting plant metabolites and their constitutions into potent extracts "Yezdex" associated with a specific disease, in this case, Diabetes. Using 15 (Fifteen) medicinal plants of Indian origin, 51 (Fifty One) unique extracts containing therapeutically relevant molecules were isolated and scientifically characterized, using advanced technologies to be used in the treatment of Diabetes. This invention also relates to an edible composition comprising of 15 (Fifteen) Indian herbal extracts which can be used as a dietary supplement/drug and also useful in lowering the glucose levels in the blood of mammals, particularly humans, suffering from Diabetes *mellitus*.

Figure 1 (A-F): Representative mass spectra of methanolic extracts (AGT_D_Gs_Ml_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05) and chloroform extracts (AGT_D_Gs_ML_03, AGT_D_Tfg_Se_03, and AGT_D_Ej_Se_03)

Fig. 1A

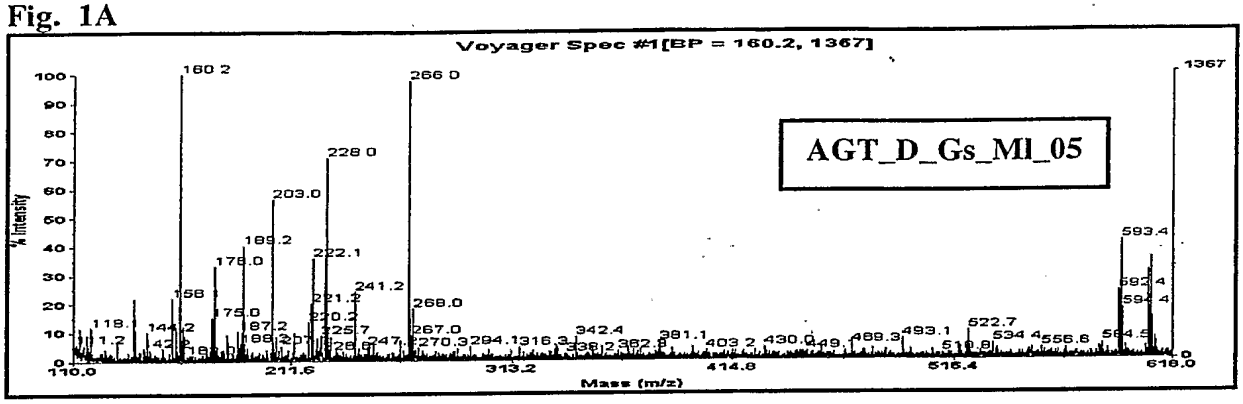


Fig. 1B

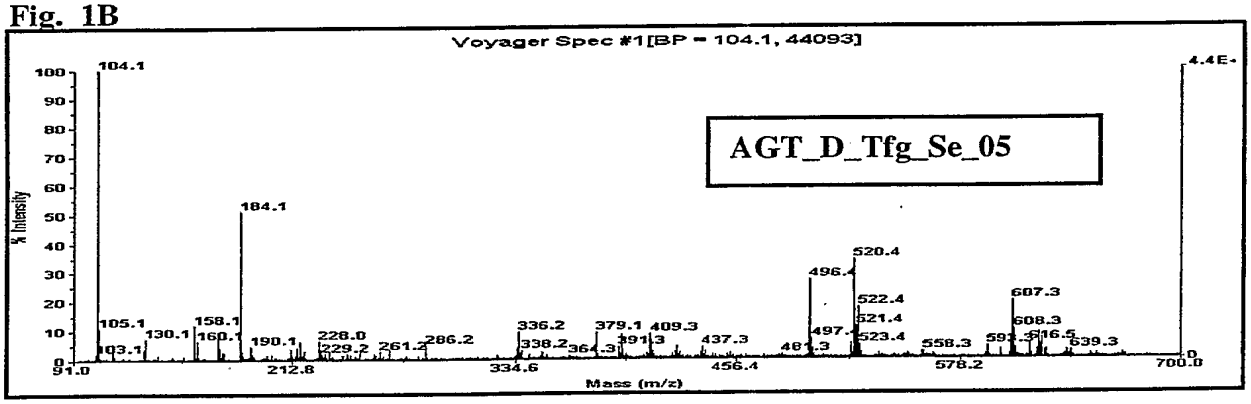


Fig. 1C

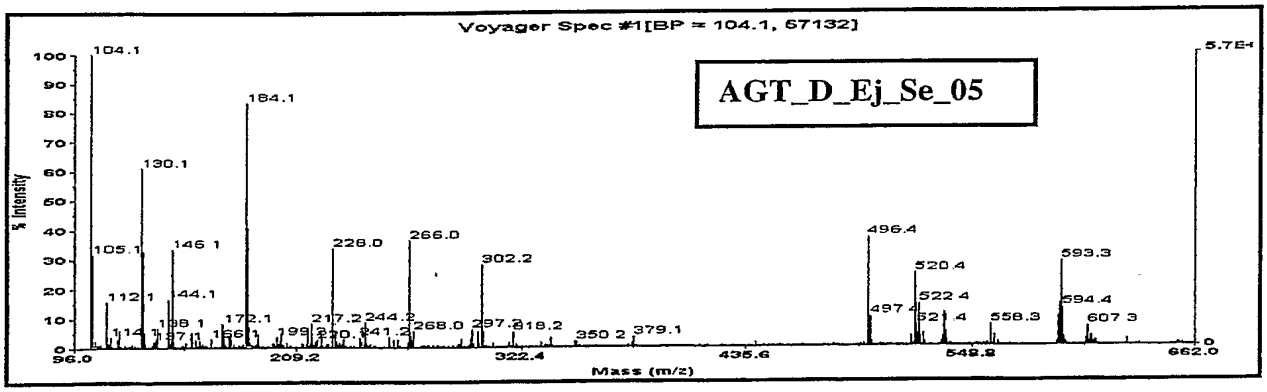


Fig. 1D

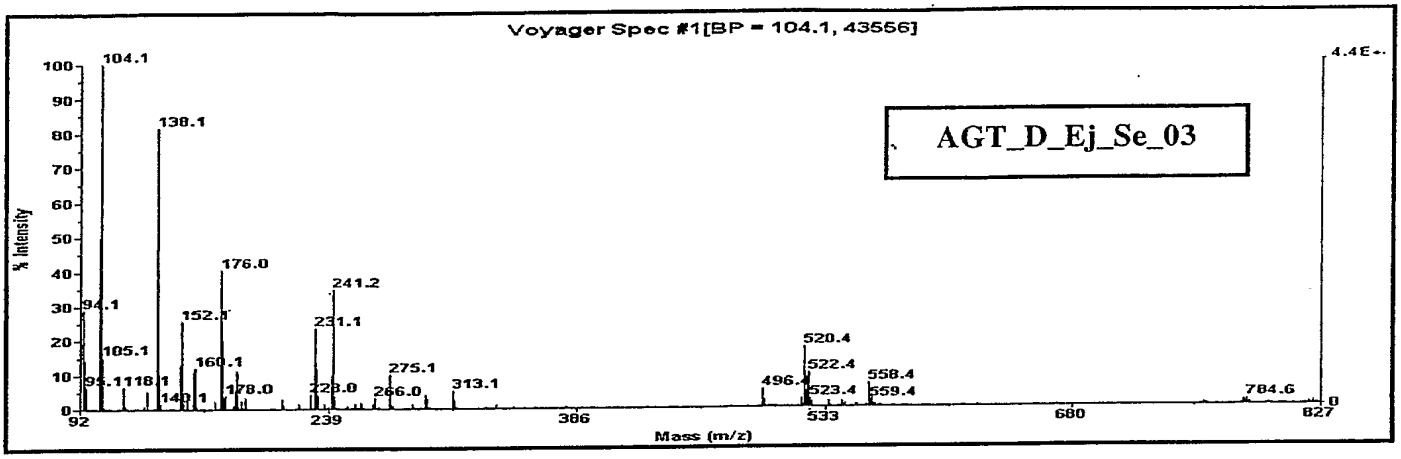


Fig. 1E

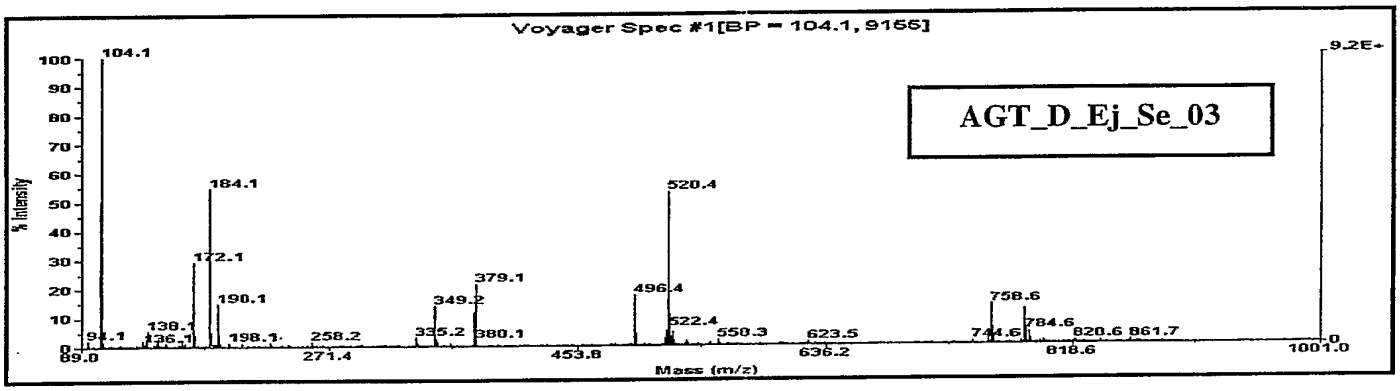


Fig. 1 F

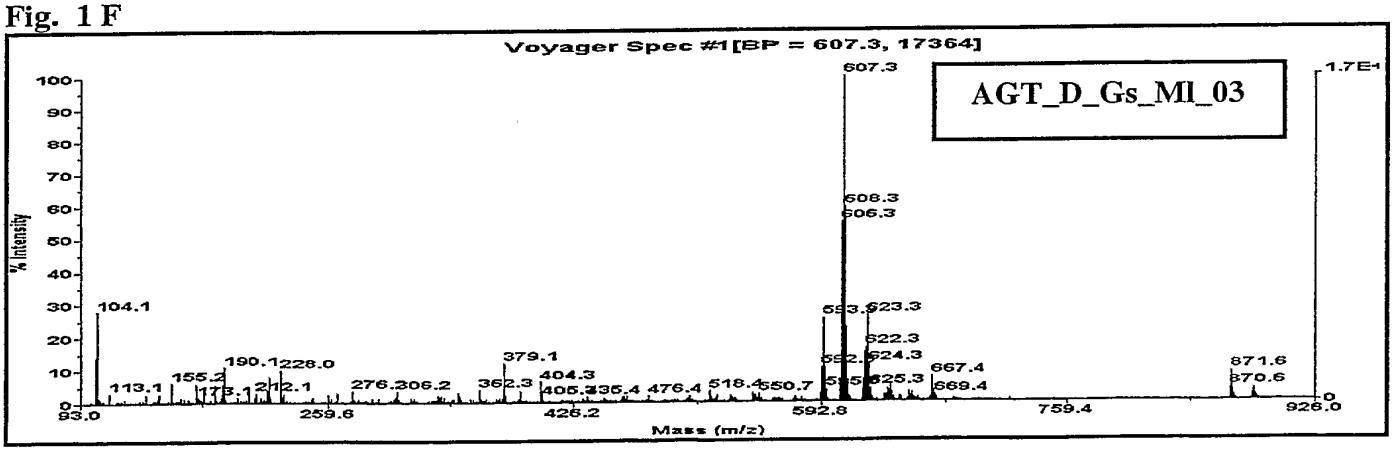
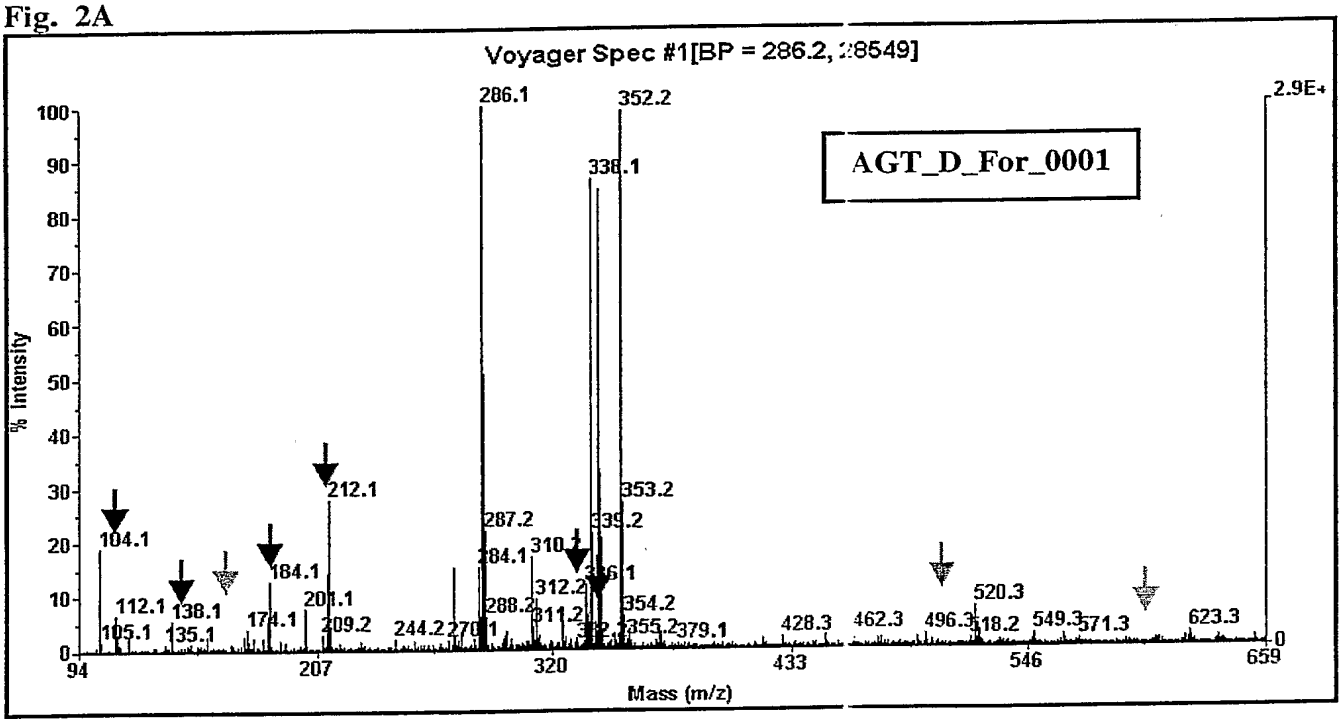


Figure 2 (A-E): Representative mass spectra of the comparative mass spectrometric analysis between the chloroform fractions of the formulation, AGT_D_For_0001_03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_03, AGT_D_Ej_Se_03, AGT_D_Tfg_Se_03 and AGT_D_Gs_MI_03.

Fig. 2A



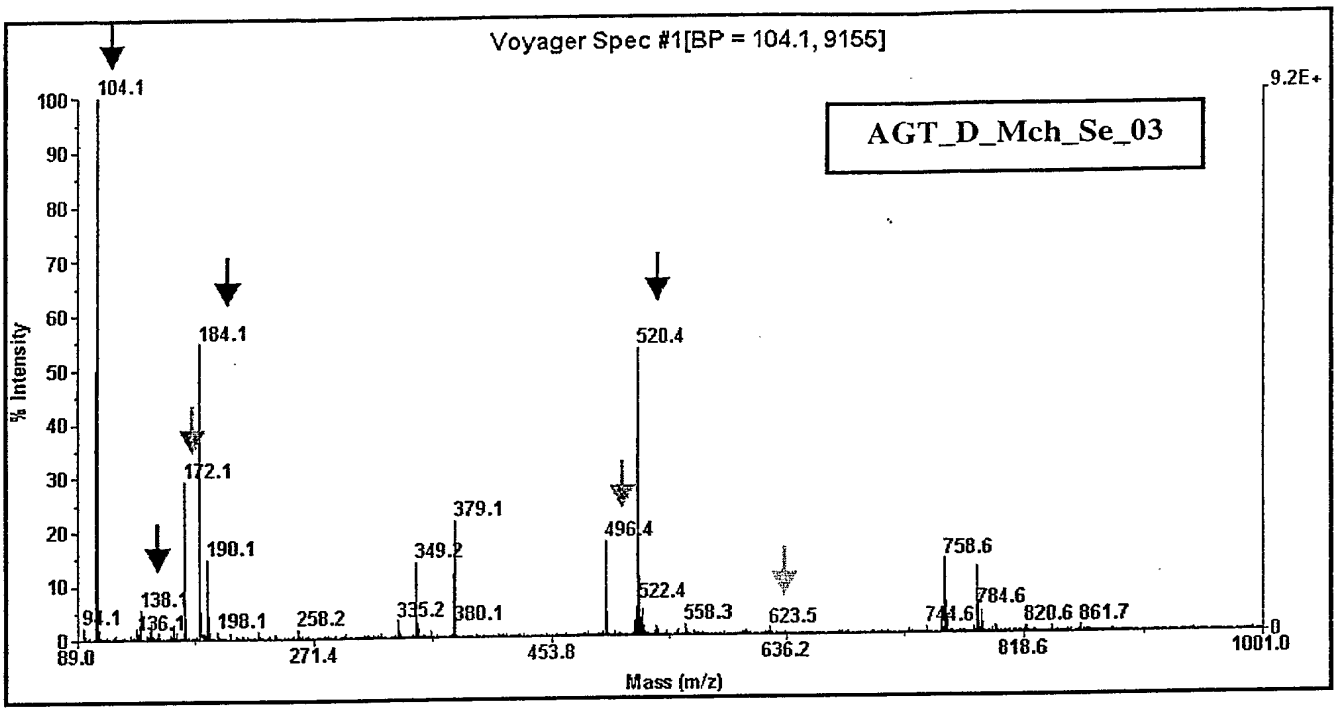

Voyager Spec #1[BP = 104.1, 9155]
AGT_D_Mch_Se_03
% Intensity
Mass (m/z)
104.1
184.1
520.4
172.1
379.1
496.4
190.1
349.2
758.6
138.1
522.4
784.6
94.1
136.1
198.1
258.2
335.2
380.1
558.3
623.5
744.6
820.6
861.7
9.2E+
89.0
271.4
453.8
636.2
818.6
1001.0

Fig. 2 B

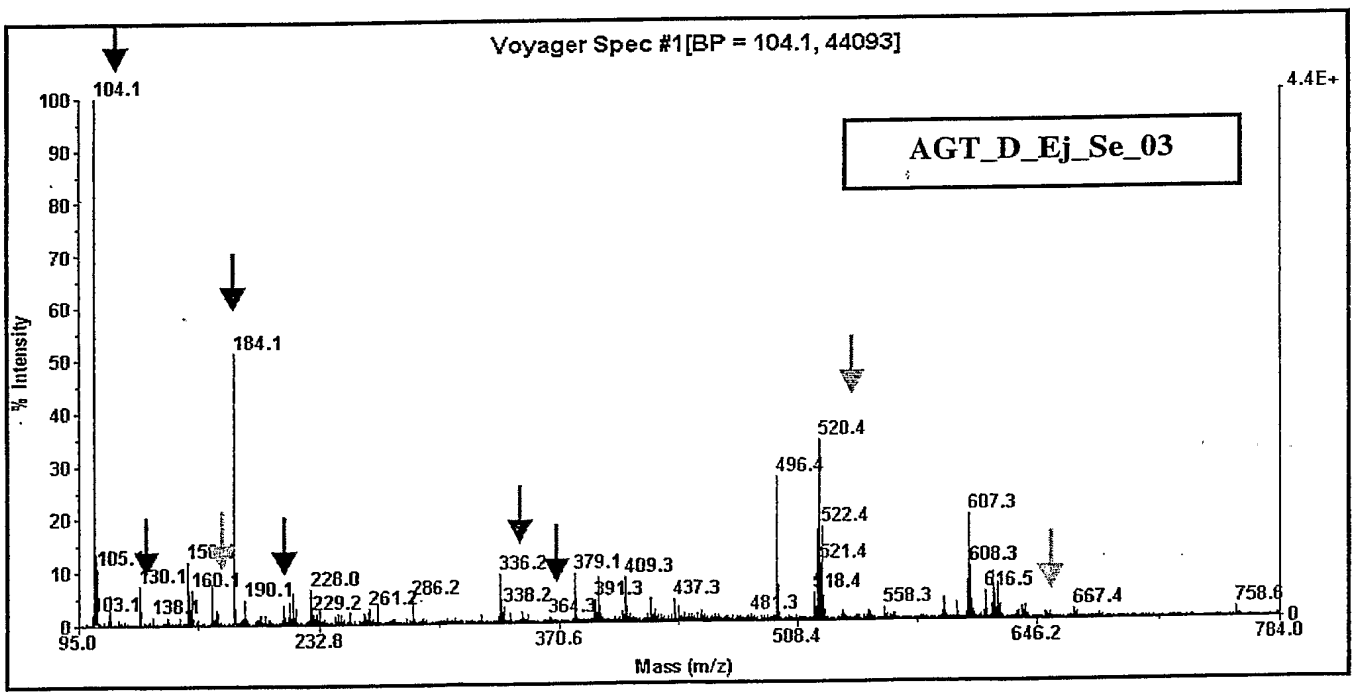

Voyager Spec #1[BP = 104.1, 44093]
AGT_D_Ej_Se_03
% Intensity
Mass (m/z)
104.1
184.1
520.4
496.4
522.4
607.3
105.1
150.1
336.2
379.1
409.3
521.4
608.3
130.1
160.1
190.1
228.0
286.2
338.2
391.3
437.3
518.4
616.5
667.4
758.6
103.1
138.1
229.2
261.2
364.3
481.3
558.3
4.4E+
95.0
232.8
370.6
508.4
646.2
784.0

Fig. 2 C

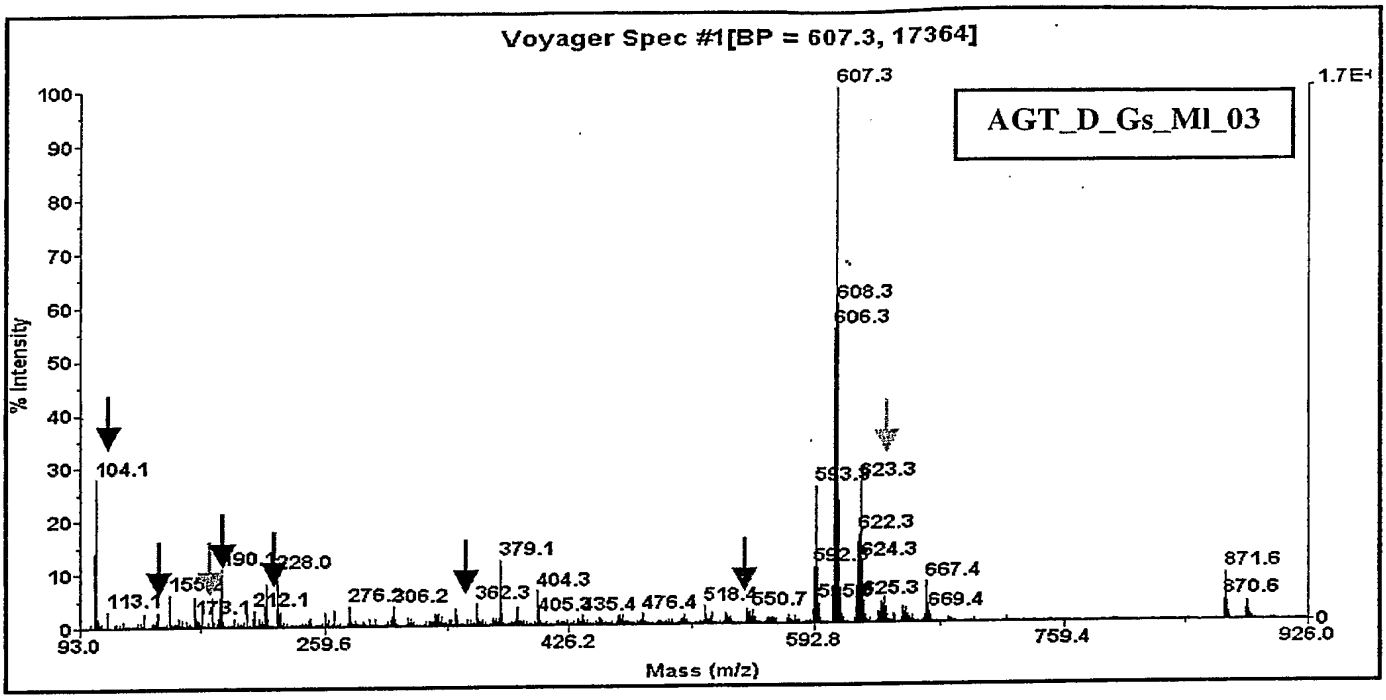

Voyager Spec #1[BP = 607.3, 17364]
AGT_D_Gs_Ml_03
% Intensity
Mass (m/z)
607.3
608.3
606.3
104.1
623.3
622.3
624.3
379.1
667.4
871.6
870.6
669.4
404.3
228.0
212.1
276.2
306.2
362.3
405.3
435.4
476.4
518.4
550.7
113.1
155.1
173.1
93.0
259.6
426.2
592.8
759.4
926.0

Fig. 2 D

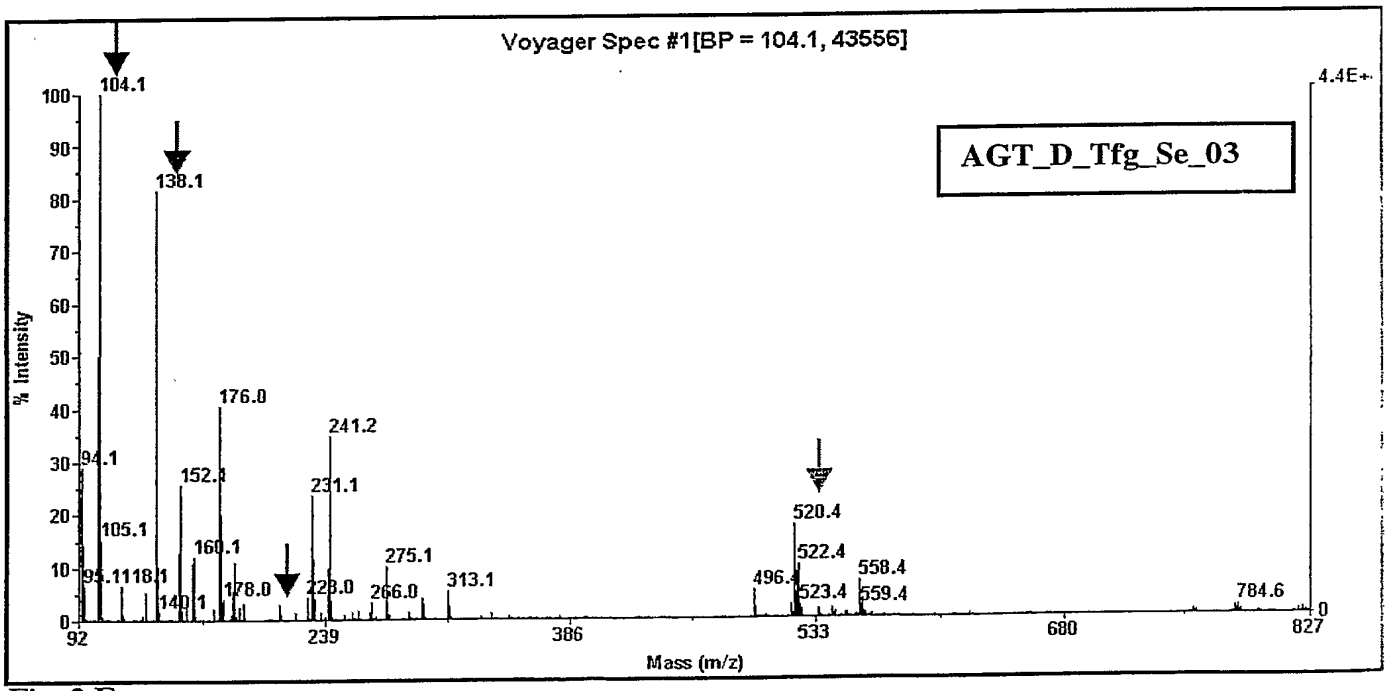

Voyager Spec #1[BP = 104.1, 43556]
AGT_D_Tfg_Se_03
% Intensity
Mass (m/z)
104.1
138.1
176.0
241.2
231.1
520.4
522.4
558.4
559.4
784.6
94.1
152.1
105.1
160.1
275.1
313.1
178.0
266.0
496.4
523.4
92
239
386
533
680
827

Fig. 2 E

Figure 3 (A-E) Representative mass spectra of the comparative mass spectrometric analysis between the chloroform –methanol fractions of the formulation, AGT_D_For_0001_05:03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_)5:03, AGT_D_Ej_Se_)5:03, AGT_D_Tfg_Se_05:03 and AGT_D_Gs_Ml_05:03.

Fig. 3 A

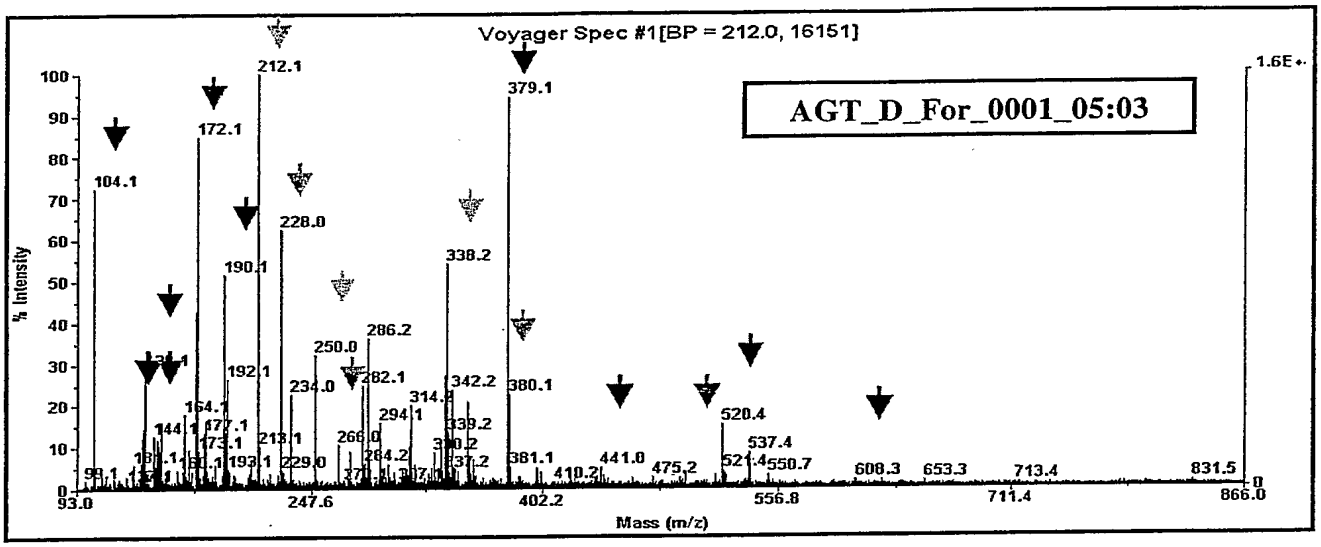


Fig. 3 B

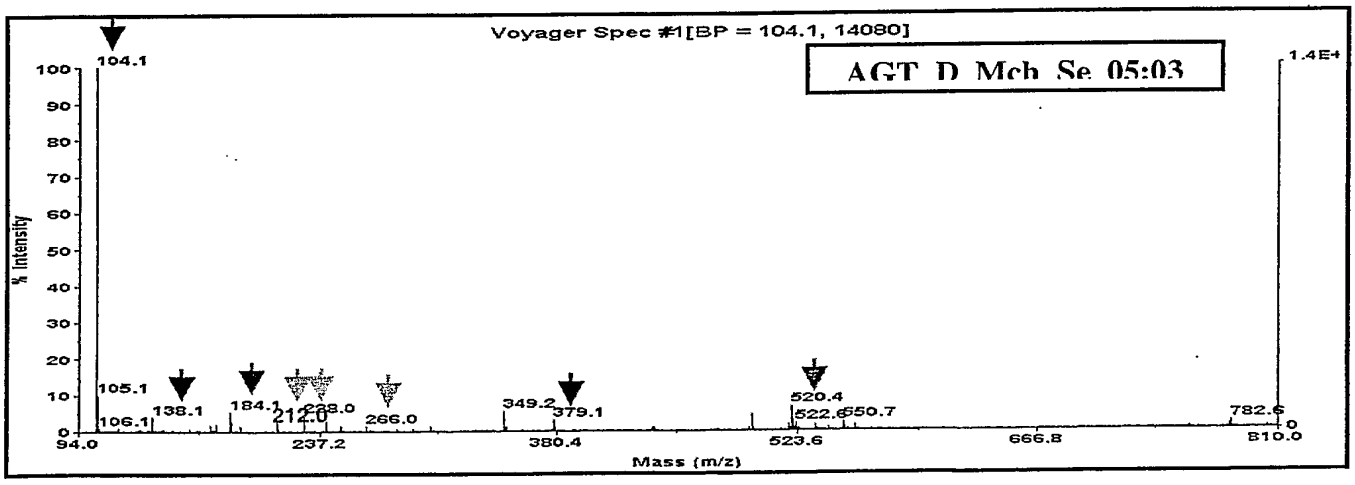


Fig. 3C

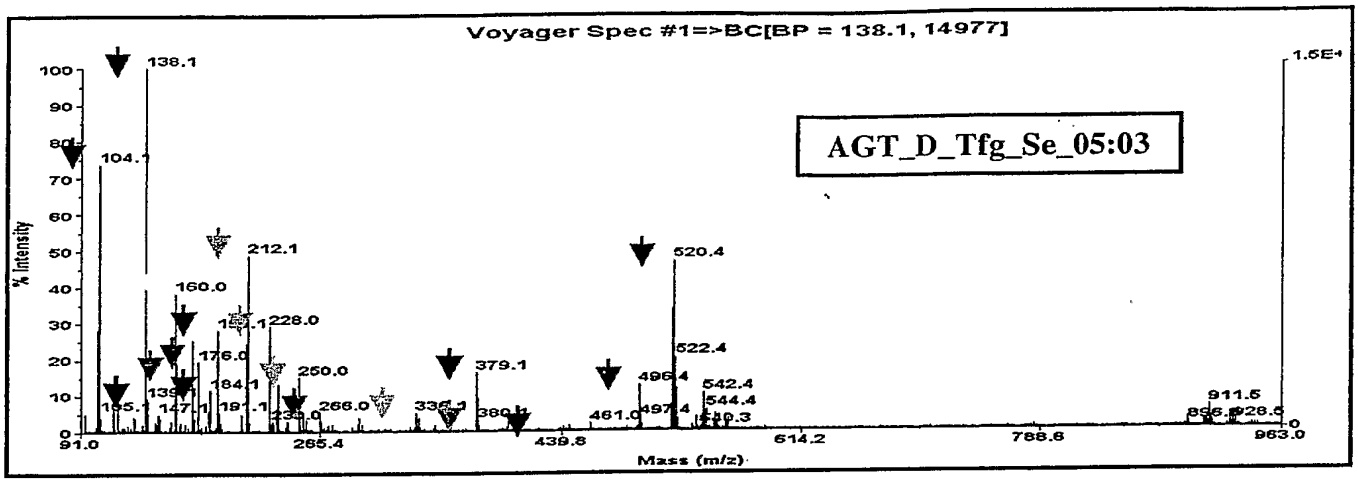

Voyager Spec #1=>BC[BP = 138.1, 14977]
AGT_D_Tfg_Se_05:03
% Intensity
Mass (m/z)
138.1
104.1
212.1
160.0
228.0
520.4
379.1
522.4
250.0
496.4
542.4
544.4
911.5
91.0
265.4
439.8
614.2
788.6
963.0

Fig. 3 D

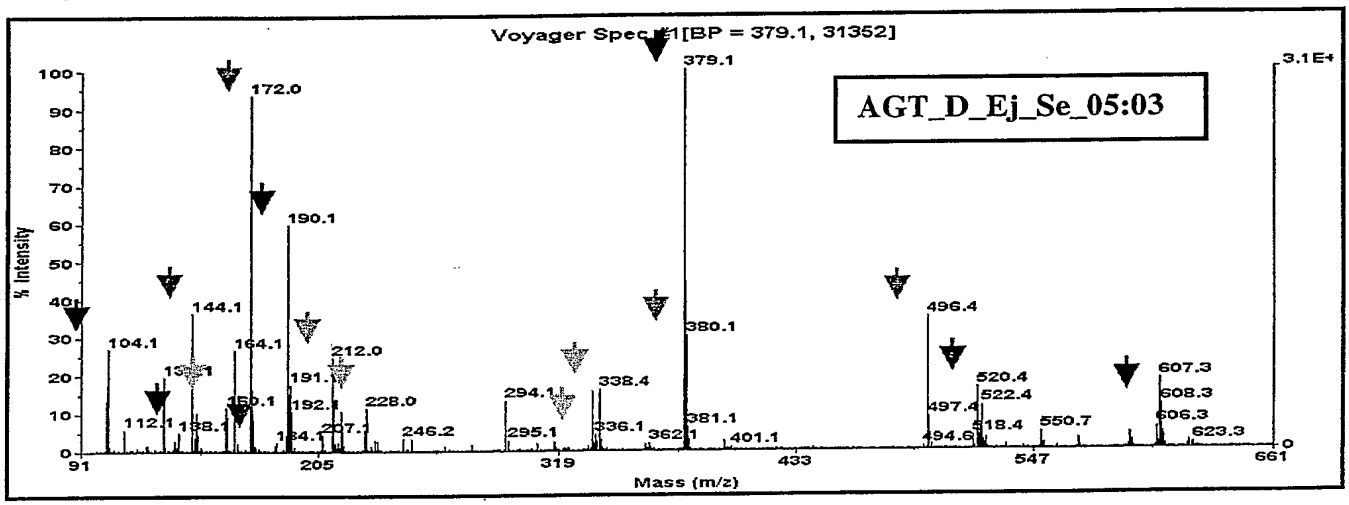

Voyager Spec #1[BP = 379.1, 31352]
AGT_D_Ej_Se_05:03
% Intensity
Mass (m/z)
379.1
172.0
190.1
144.1
496.4
104.1
164.1
212.0
380.1
338.4
520.4
522.4
607.3
608.3
606.3
294.1
228.0
497.4
550.7
623.3
91
205
319
433
547
661

Fig. 3E

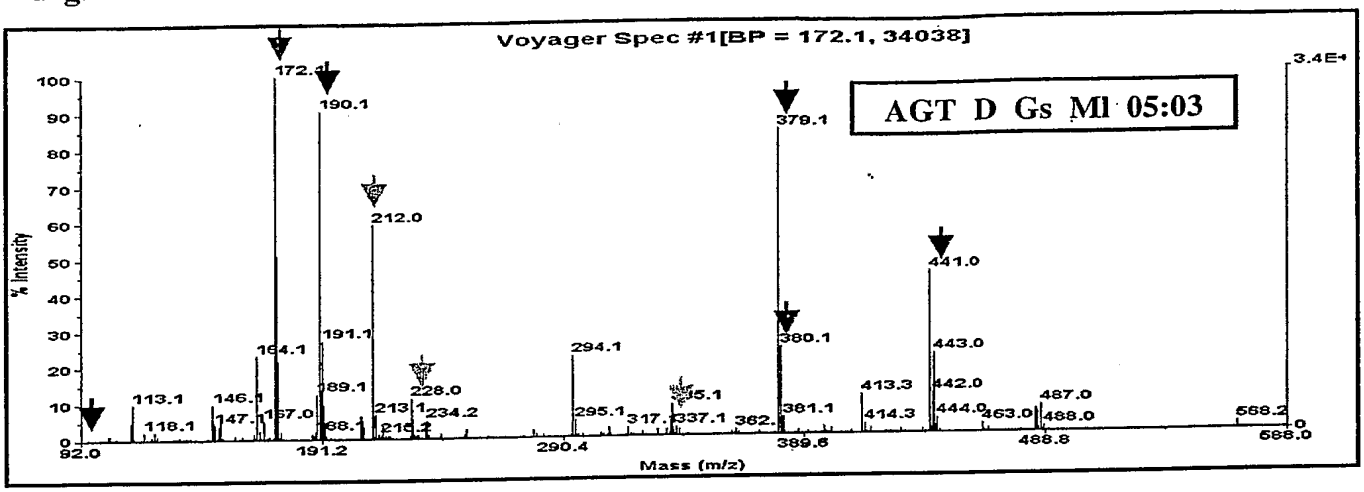

Voyager Spec #1[BP = 172.1, 34038]
AGT D Gs Ml 05:03
% Intensity
Mass (m/z)
172.1
190.1
212.0
379.1
441.0
3.4E+
100
90
80
70
60
50
40
30
20
10
0
92.0
191.2
290.4
389.6
488.8
588.0

Figure 4 (A-E) Representative mass spectra of the comparative mass spectrometric analysis between the methanol fractions of the formulation, AGT_D_For_0001_05 and the methanol extracts of a few of its constituents, AGT_D_Mch_Se_05, AGT_D_Ej_Se_05, AGT_D_Tfg_Se_05 and AGT_D_Gs_Ml_05.

Fig. 4 A

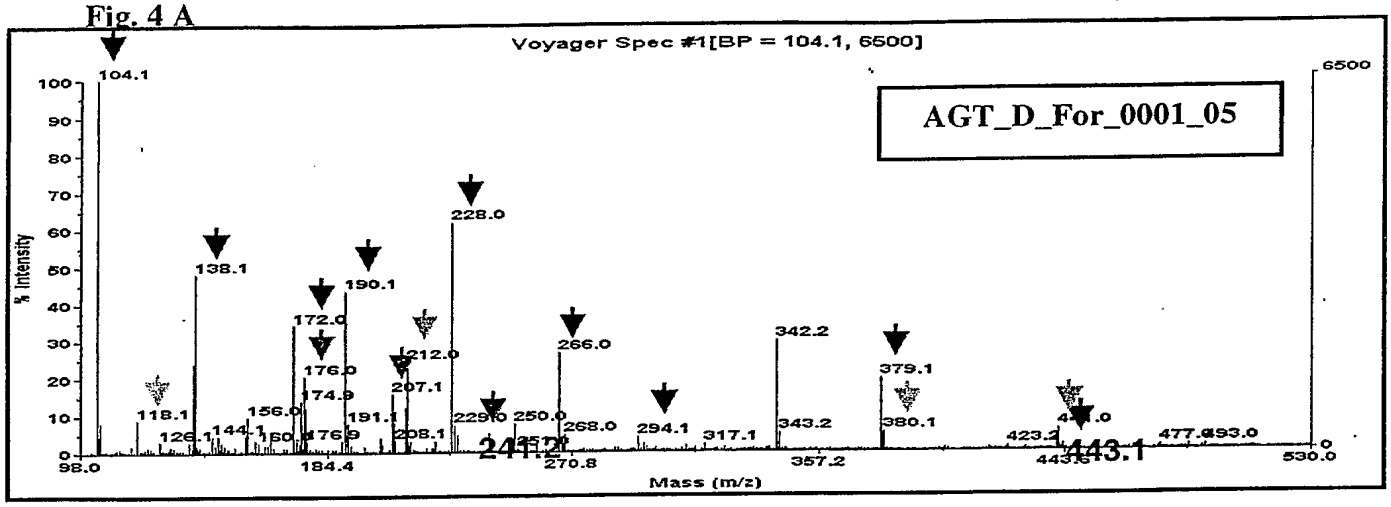


Fig. 4 B

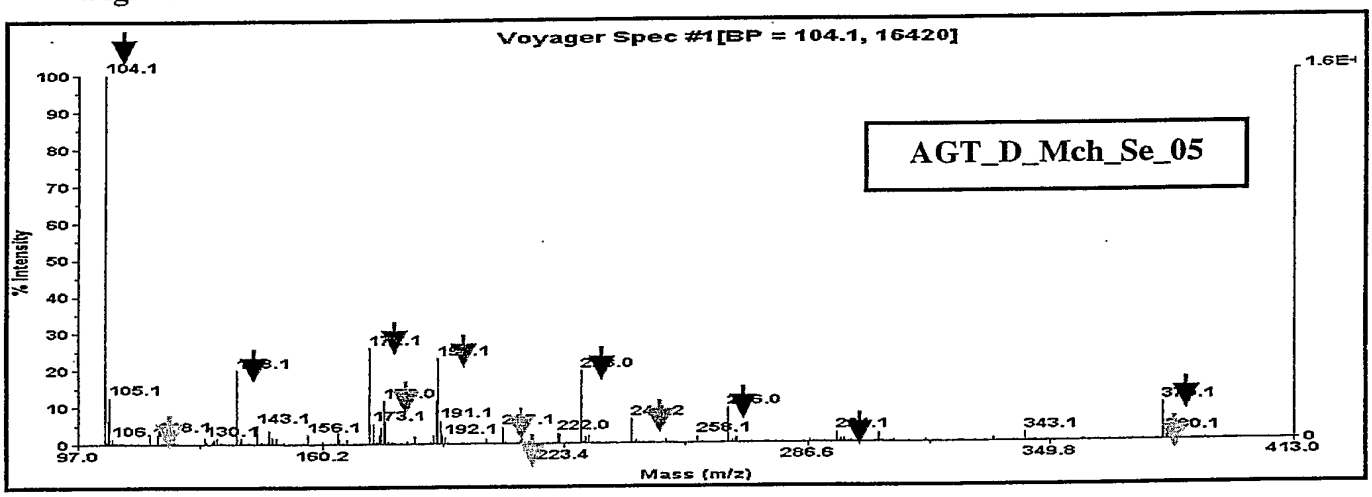
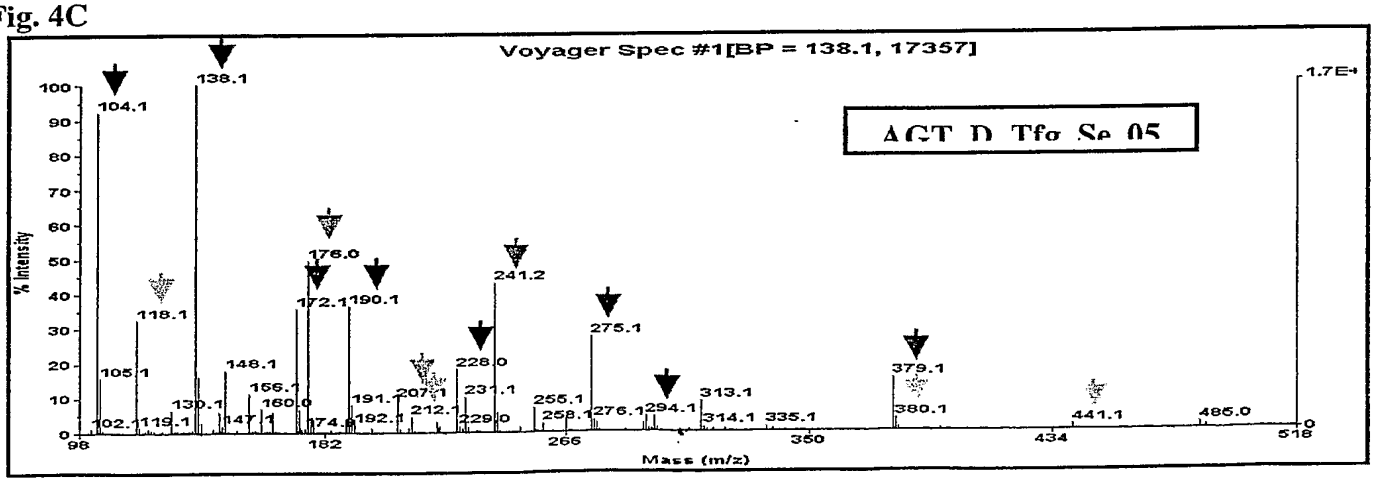


Fig. 4C



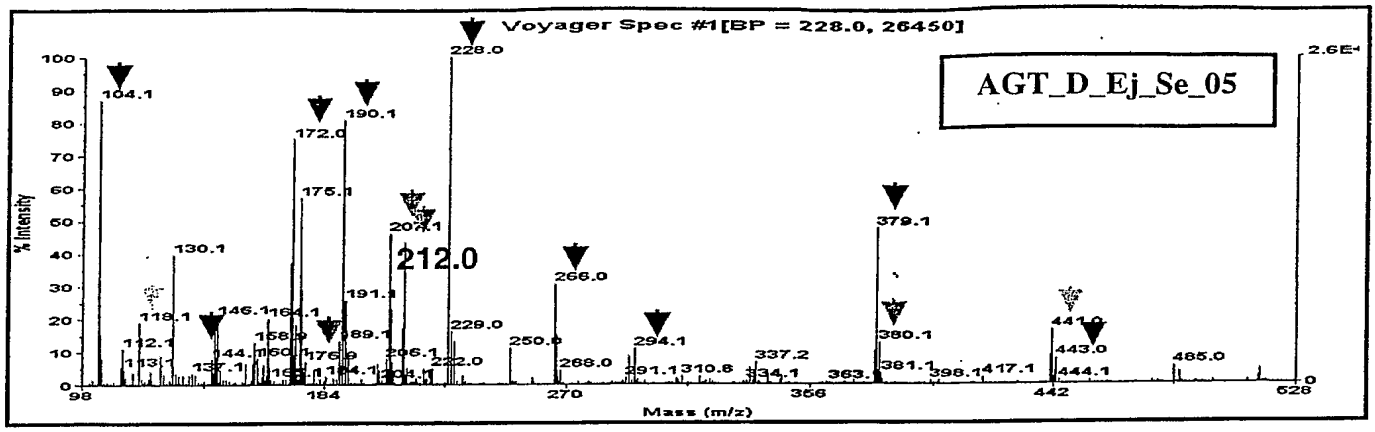

Voyager Spec #1[BP = 228.0, 26450]
AGT_D_Ej_Se_05
% Intensity
Mass (m/z)
212.0

Fig. 4 D

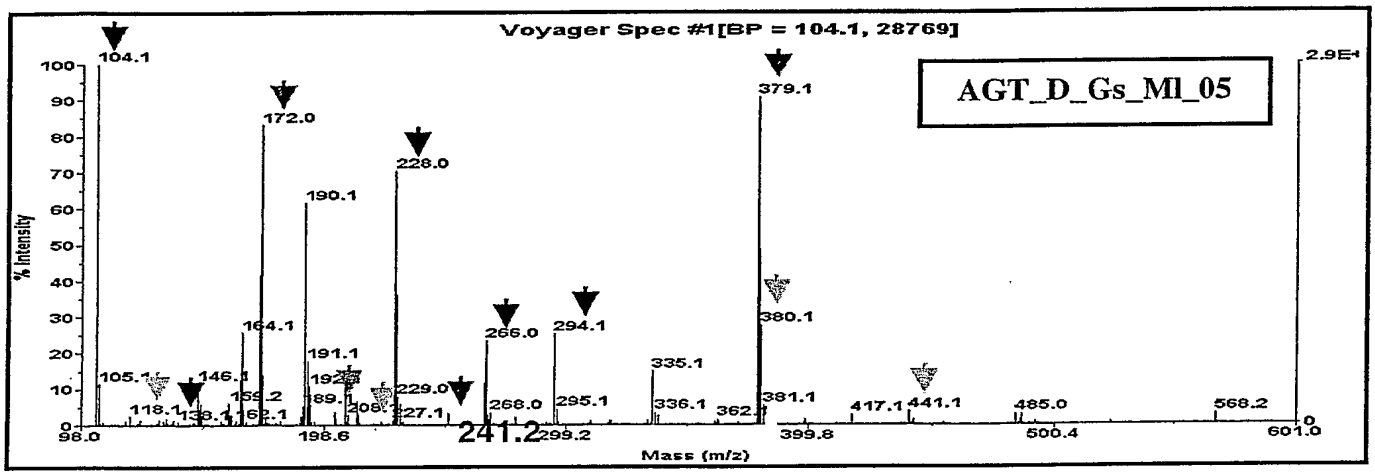
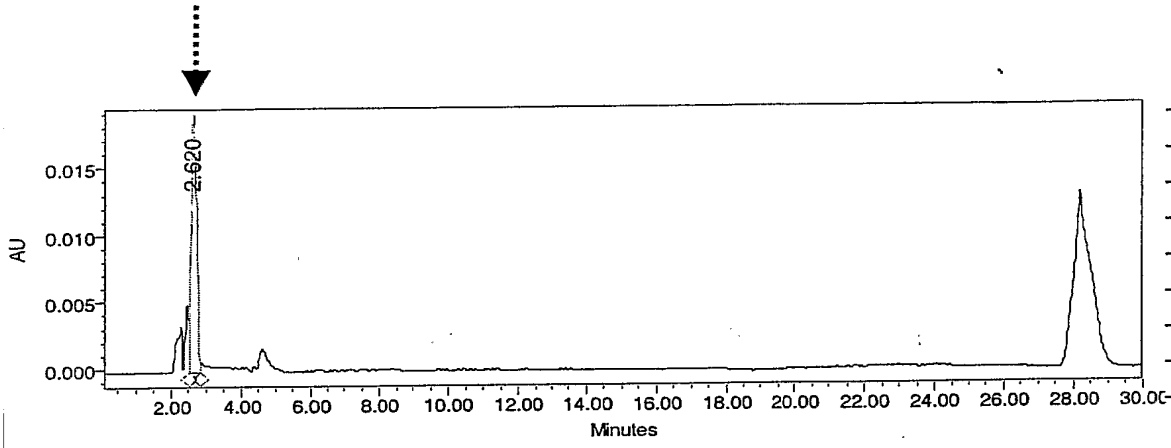

Voyager Spec #1[BP = 104.1, 28769]
AGT_D_Gs_Ml_05
% Intensity
Mass (m/z)
241.2

Fig. 4 E

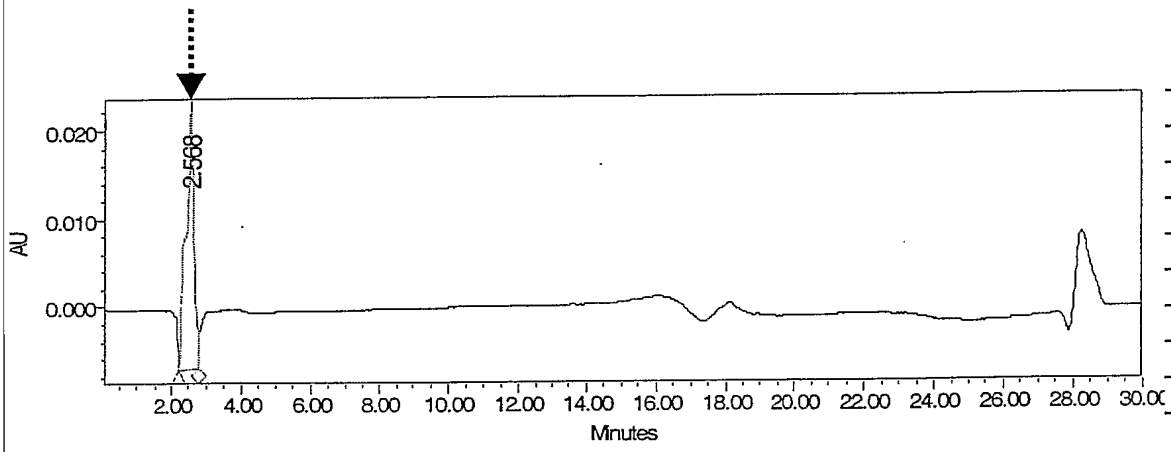
Fig 5: Chromatograms of extracts Ma_Le_Wa_01 and Ws_Ro_Wa_01 at selected wavelengths

Fig. 5A Ma_Le_Wa_01 at 580 nm



	R. Time	Lambda Max	Abs. M
1	0.107	646	0.00
2	2.073	201.9	1.76
3	**2.620**	**485**	**0.00**
4	21.014	353.6	0.00
5	23.429	190.1	0.0
6	23.813	190.1	0.42
7	28.177	190.1	0.96

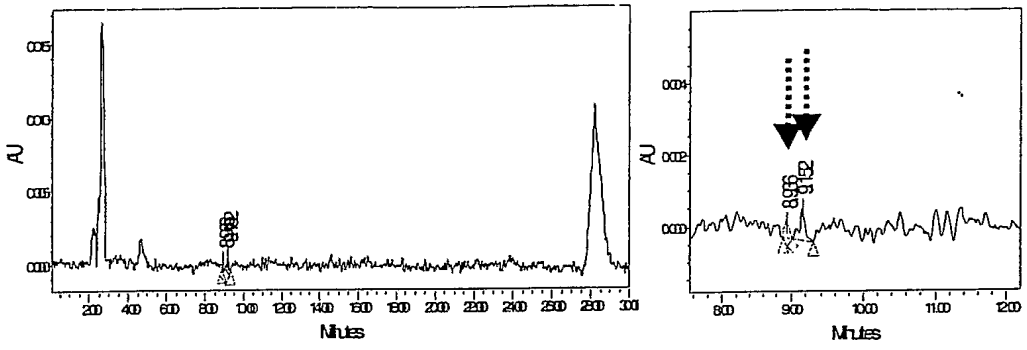
Fig. 5B Ws_Ro_Wa_01 at 580 nm



	R. Time	Lambda max	Abs. M
1	0.102	668.1	0.00
2	0.102	668.1	0.00
3	2.301	328.5	0.02
4	**2.568**	**485**	**0.00**
5	2.901	658.3	0.00
6	17.862	200.7	0.0
7	18.095	199.5	0.02
8	28.241	192.5	0.05

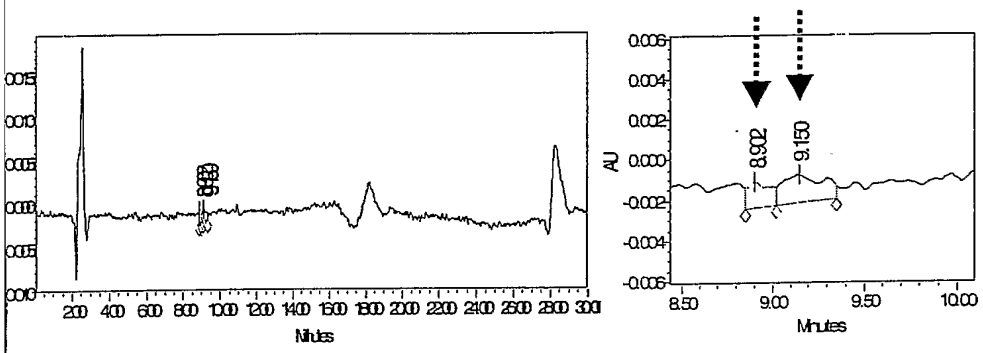
Fig. 5 (Cont'd)

Fig. 5C Ma_Le_Wa_01 at 650 nm



	R. Time	Lambda Max	Abs.
28	6.954	649.7	0.00
29	7.254	658.3	0.00
30	7.77	649.7	0.00
31	8.087	649.7	0.00
32	8.42	649.7	0.00
33	8.52	666.9	0.00
34	8.72	649.7	0.00
35	**8.936**	**660.8**	**0.00**
36	**9.152**	**660.8**	**0.00**
37	9.286	668.1	0.00
38	9.319	649.7	0.00
39	9.469	649.7	0.00
40	9.769	649.7	0.00
41	9.902	649.7	0.00
42	10.952	649.7	0.00
43	11.102	649.7	0.00
44	11.218	205.4	0.00
45	11.452	192.5	0.00
46	11.602	191.3	0.00

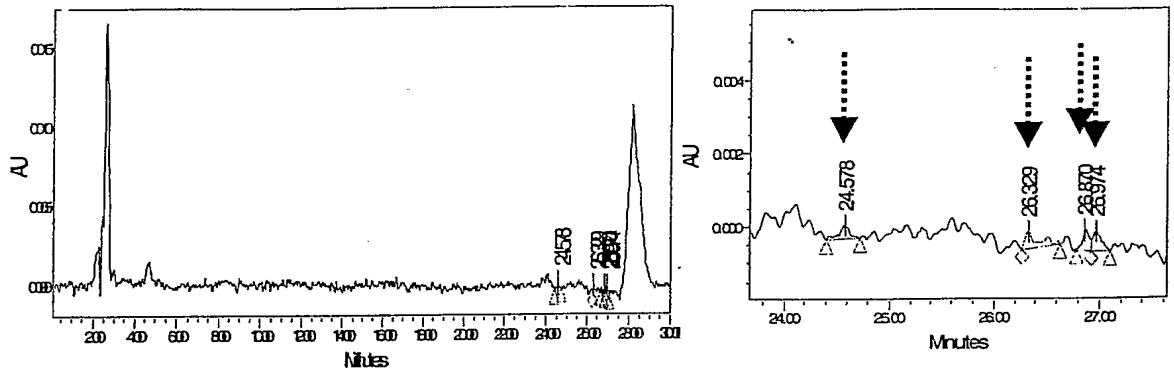
Fig. 5D Ws_Ro_Wa_01 at 650 nm



	R. Time	Lambda max	Abs. Max
38	7.649	697.5	0.00262
39	7.899	660.8	0.00248
40	8.182	660.8	0.00249
41	8.382	660.8	0.00344
42	8.532	660.8	0.00297
43	8.715	660.8	0.00326
44	**8.902**	**660.8**	**0.0029**
45	**9.150**	**660.8**	**0.00265**
46	9.265	651	0.00313
47	9.598	660.8	0.00313
48	9.715	660.8	0.00287
49	9.865	192.5	0.00876

Fig. 5 (Cont'd)

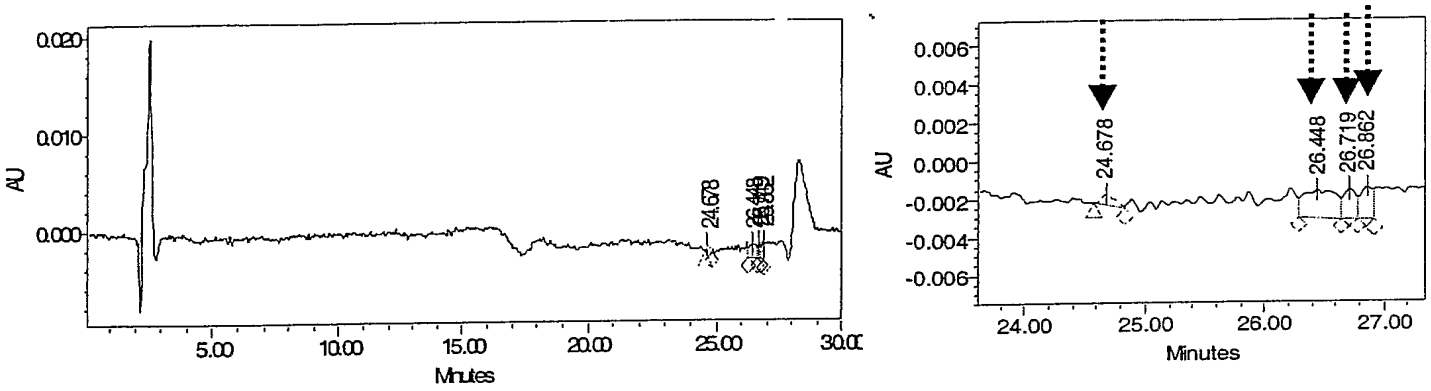
Fig. 5 E Ma_Le_Wa_01 at 690 nm



	R. Time	Lambda Max	Abs. Max
90	24.013	190.1	0.35506
91	24.212	190.1	0.41454
92	24.379	190.1	0.44123
93	**24.579**	**190.1**	**0.45064**
94	24.795	190.1	0.45579
95	24.912	190.1	0.44643
96	25.129	190.1	0.42456
97	25.279	190.1	0.40138
98	25.728	190.1	0.31581
99	25.828	190.1	0.29505
100	26.095	190.1	0.23558
101	**26.329**	**190.1**	**0.20293**
102	26.528	190.1	0.12837
103	**26.870**	**190.1**	**0.05476**
104	**26.974**	**190.1**	**0.01977**
105	27.161	192.5	0.07595
106	27.228	192.5	0.10762
109	28.56	190.1	0.0157
110	29.343	697.5	0.00093
111	29.693	651	0.0008

Fig. 5 (Cont'd)

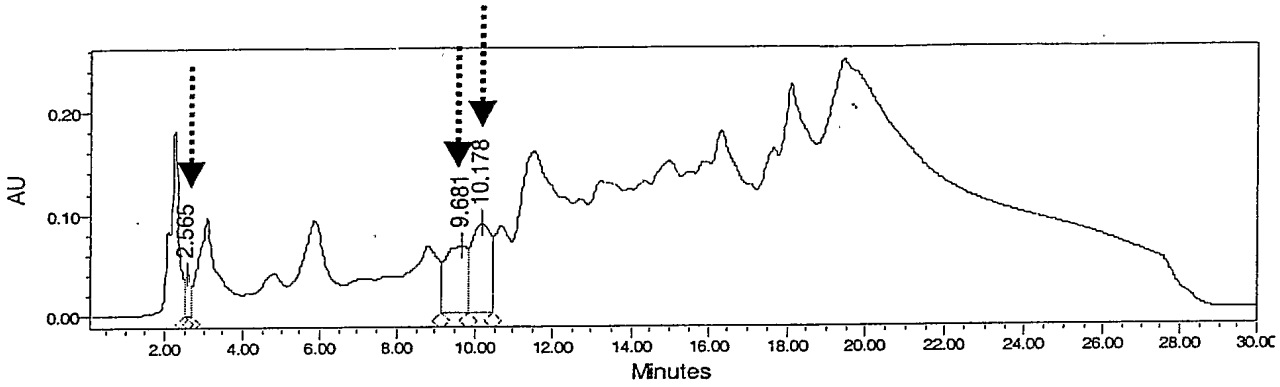
Fig. 5 F Ws_Ro_Wa_01 at 690 nm



	R. Time	Lambda max	Abs. Max
88	23.276	199.5	0.01191
89	23.709	193.6	0.00354
90	23.909	192.5	0.00157
91	24.159	191.3	0.00458
92	**24.678**	**190.1**	**0.00477**
93	24.776	197.2	0.00349
94	24.875	194.8	0.00495
95	25.075	190.1	0.00559
96	25.159	194.8	0.00554
97	25.342	193.6	0.0023
98	25.775	194.8	0.00623
99	25.875	197.2	0.00387
100	26.058	194.8	0.00458
101	26.308	690.2	0.00113
102	**26.448**	**190.1**	**0.00492**
103	**26.719**	**190.1**	**0.00528**
104	**26.862**	**190.1**	**0.00627**
105	27.658	197.2	0.09525
106	28.224	192.5	0.04129
107	29.257	191.3	0.00285
109	29.624	190.1	0.0024
110	29.79	191.3	0.00104

Fig 6: Chromatograms of extracts Cr_Ro_Et_01 and Ws_Ro_Et_01 at selected wavelengths

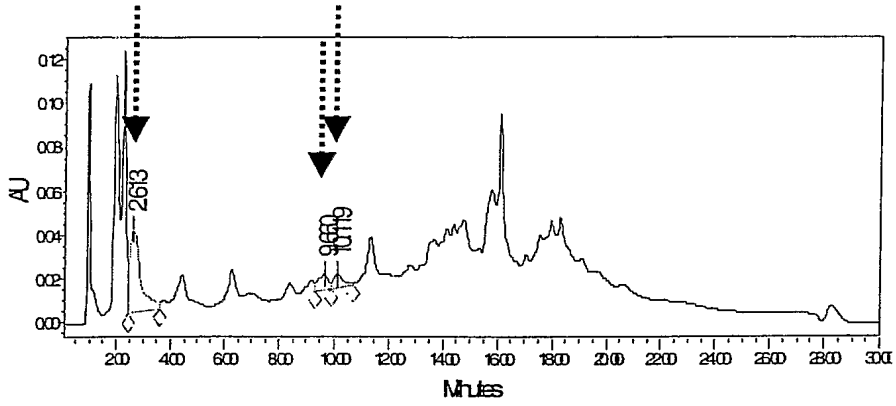
Fig. 6 A Cr_Ro_Et_01 at 270 nm



	R. Time	Lambda Max.	Abs. Max
1	2.087	199.5	0.50854
2	2.254	234.8	1.9173
3	2.565	192.5	0.33016
4	3.087	232.4	0.64575
5	4.22	194.8	0.11512
6	4.82	192.5	0.19056
7	5.87	192.5	0.4551
8	7.236	193.6	0.49654
9	7.736	193.6	0.59015
10	8.802	193.6	0.79979
11	9.681	193.6	0.83845
12	10.178	193.6	0.94701
13	10.685	194.8	0.98703
14	11.518	194.8	1.31397
15	12.285	194.8	1.24312
16	12.701	194.8	1.26801
17	13.218	194.8	1.35583
18	13.434	196	1.35745
19	13.951	196	1.39184
20	14.334	196	1.45647
21	14.967	196	1.52994
22	15.484	196	1.50795
23	15.851	197.2	1.55668
24	15.967	197.2	1.56793
25	16.317	197.2	1.62721
26	17.65	199.5	1.84688
27	18.1	199.5	2.01099
28	19.417	199.5	2.00576

Fig.6 (Cont'd)

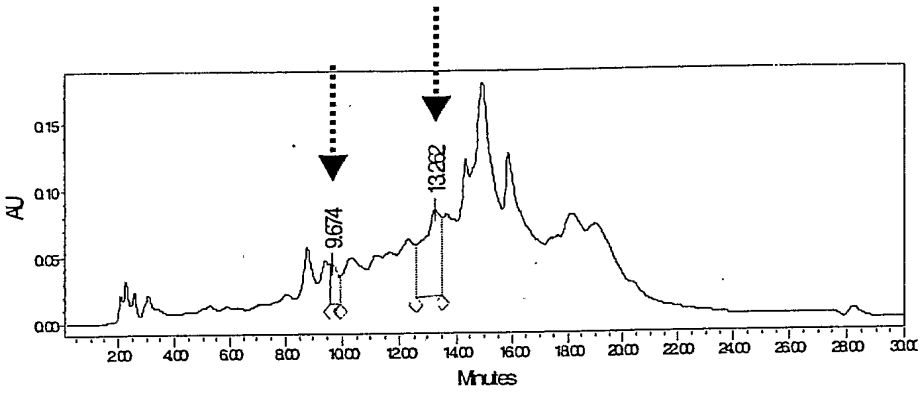
Fig. 6 B Ws_Ro_Et_01 at 270 nm



	R. Time	Lambda Max	Abs. Max
1	0.115	190.1	0.00028
2	0.948	197.2	0.41888
3	1.947	197.2	0.68039
4	2.231	193.6	0.79799
5	**2.613**	**192.5**	**0.19402**
6	3.73	200.7	0.01838
7	4.397	198.3	0.09547
8	6.247	194.8	0.18099
9	6.996	192.5	0.01873
10	8.379	194.8	0.08258
11	9.179	193.6	0.04649
12	**9.660**	**193.6**	**0.0514**
13	**10.119**	**193.6**	**0.05152**
14	10.696	193.6	0.03452
15	11.345	194.8	0.0667
16	11.929	192.5	0.02847
17	12.812	197.2	0.09257
18	13.295	196	0.14591
19	13.662	197.2	0.32274
20	13.912	197.2	0.26242
21	14.111	197.2	0.37587
22	14.378	200.7	0.95824
23	14.745	224.2	1.1217
24	15.328	197.2	0.41881
25	15.761	199.5	0.85325
26	16.078	197.2	0.63455
27	17.044	198.3	0.4413
28	17.561	200.7	0.57871
29	17.961	200.7	0.6471
30	18.294	200.7	0.72115
31	19.044	200.7	0.50734
32	19.644	200.7	0.38701
33	20.61	200.7	0.20326
34	28.242	192.5	0.05557

Fig.6 (Cont'd)

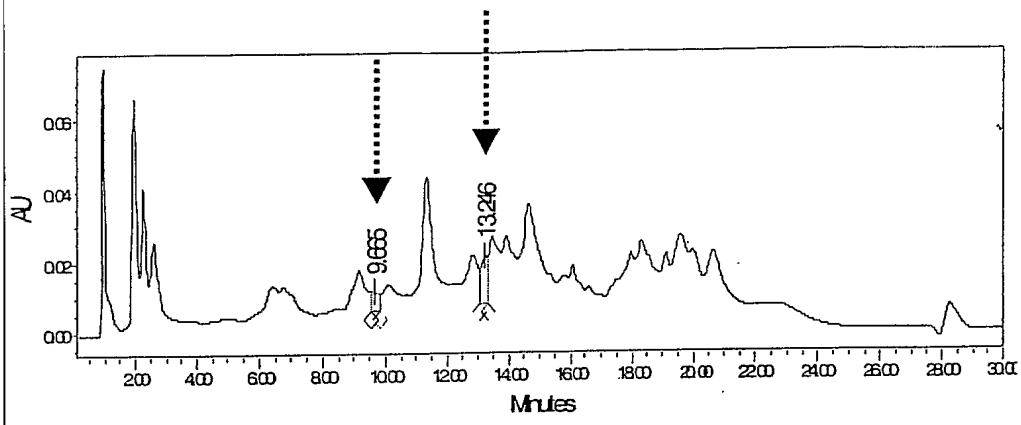
Fig. 6 C Cr_Ro_Et_01 at 330 nm



	R. Time	Lambda Max.	Abs. Max
1	2.07	200.7	0.4866
2	2.254	234.8	1.88548
3	2.57	192.5	0.21094
4	3.053	232.4	0.77904
5	3.387	205.4	0.10594
6	4.087	286.9	0.00364
7	4.353	290.4	0.00329
8	5.27	286.9	0.00784
9	5.853	246.6	0.18044
10	7.119	193.6	0.04334
11	8.036	193.6	0.09153
12	8.785	193.6	0.2405
13	9.435	193.6	0.20392
14	9.674	193.6	0.21604
15	10.335	193.6	0.29389
16	11.185	194.8	0.34762
17	11.668	194.8	0.50063
18	12.335	194.8	0.3846
19	13.262	194.8	0.4188
20	13.651	194.8	0.37805
21	14.334	196	0.36579
22	14.934	196	0.3696
23	15.851	196	0.29167
24	17.4	197.2	0.18577
25	17.667	201.9	0.31291
26	18.1	201.9	0.48327
27	19	199.5	0.17848
28	28.215	485	0.01591

Fig.6 (Cont'd)

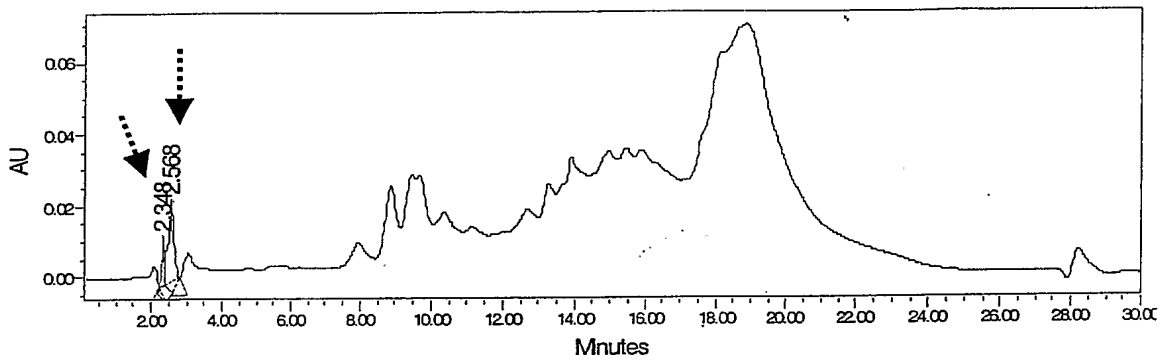
Fig. 6D Ws_Ro_Et_01 at 330 nm



	R. Time	Lambda Max	Abs. Max
1	0.115	190.1	0.00028
2	0.948	197.2	0.40018
3	1.931	196	0.63299
4	2.247	193.6	0.77062
5	2.581	192.5	0.17303
6	6.43	192.5	0.04709
7	6.747	192.5	0.02341
8	8.413	194.8	0.08999
9	9.163	193.6	0.08332
10	9.665	193.6	0.10695
11	10.112	193.6	0.12405
12	11.329	194.8	0.19829
13	12.162	194.8	0.16632
14	12.812	194.8	0.2143
15	13.246	194.8	0.21948
16	13.462	194.8	0.22538
17	13.912	194.8	0.24628
18	14.628	217.1	1.0283
19	15.811	228.9	0.65343
20	16.094	231.3	0.47052
21	16.628	194.8	0.02992
22	17.477	201.9	0.33393
23	17.961	201.9	0.34255
24	18.311	201.9	0.48014
25	19.127	201.9	0.2821
26	19.594	200.7	0.23022
27	19.993	200.7	0.1926
28	20.643	200.7	0.11251
29	28.242	192.5	0.05651

Fig.6 (Cont'd)

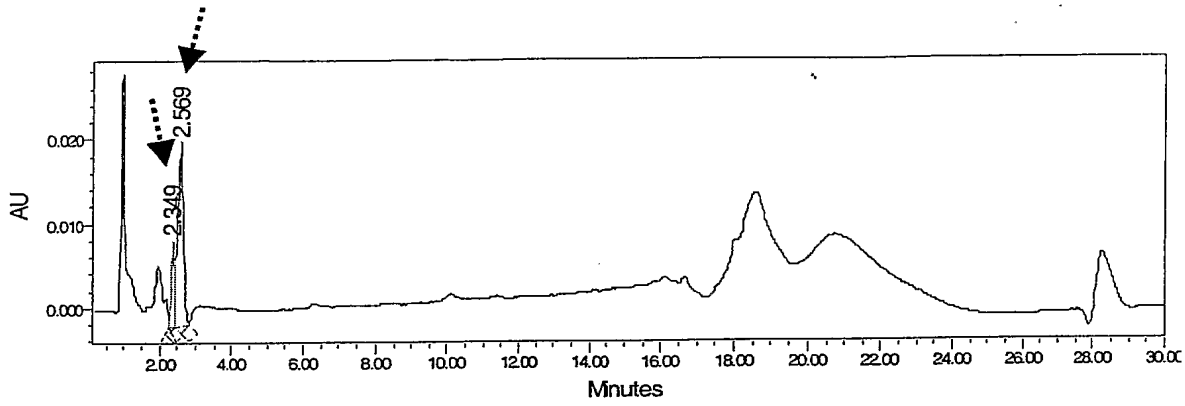
Fig. 6E Cr_Ro_Et_01 at 400 nm



	R. Time	Lambda Max.	Abs. Max
1	2.104	658.3	0.00939
2	2.348	485	0.02139
3	2.568	485	0.0388
4	3.037	233.6	0.45082
5	4.753	252.5	0.01205
6	5.42	420.8	0.00098
7	7.952	412.3	0.00628
8	8.869	193.6	0.08148
9	9.452	250.1	0.03225
10	9.669	389.4	0.02281
11	10.368	193.6	0.04742
12	11.135	193.6	0.01907
13	11.985	223	0.04917
14	12.685	194.8	0.00692
15	13.268	194.8	0.06038
16	13.901	196	0.03483
17	14.951	328.5	0.10475
18	15.384	328.5	0.02544
19	15.834	328.5	0.04568
20	18.217	203	0.64432
21	18.85	201.9	0.45036
22	28.215	485	0.01612

Fig.6 (Cont'd)

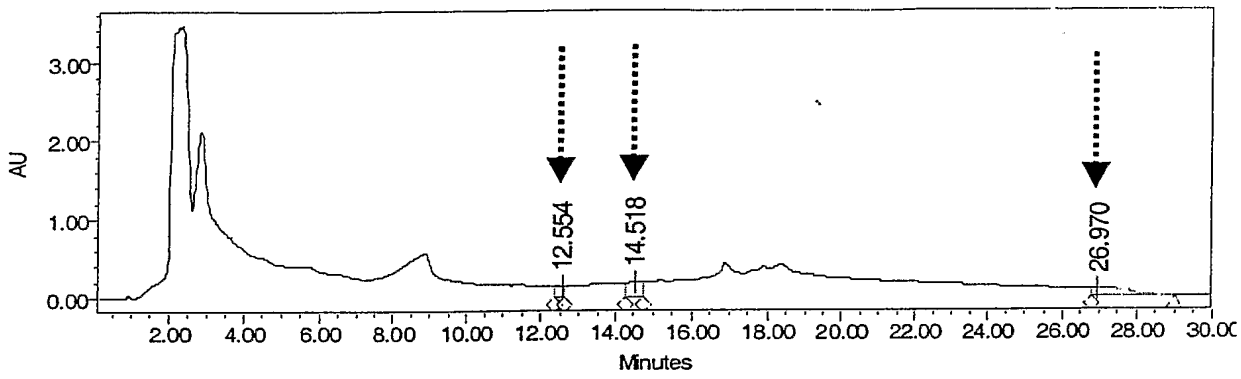
Fig. 6F Ws_Ro_Et_01 at 400 nm



	R. Time	Lambda Max	Abs. Max
1	0.115	190.1	0.00028
2	0.948	199.5	0.32804
3	1.764	485	0.00682
4	1.947	206.6	0.16692
5	2.349	485	0.02023
6	2.569	485	0.04148
7	3.097	485	0.00834
8	6.263	226.5	0.03186
9	10.079	192.5	0.00543
10	15.811	230.1	0.52905
11	16.078	232.4	0.40994
12	16.644	357.2	0.00291
13	17.994	200.7	0.25105
14	18.544	200.7	0.26132
15	20.743	200.7	0.10022
16	28.242	192.5	0.05651

Fig 7: Chromatograms of extracts Ej_Se_Me_01 and Tt_Fr_Me_01 at selected wavelengths

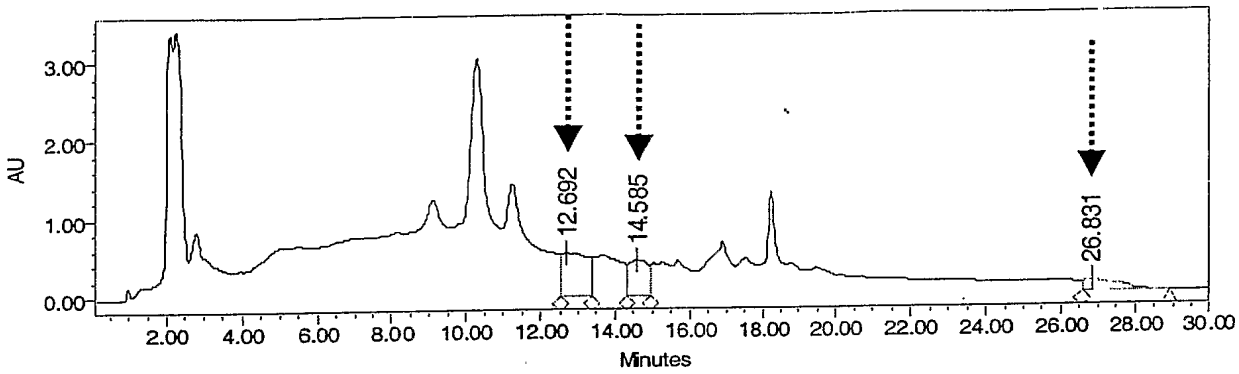
Fig. 7A Ej_Se_Me_01 at 210 nm



	R. Time	Lambda Max	Abs. Max
1	0.111	664.4	0.00017
2	0.928	197.2	0.0545
3	2.211	237.2	4.18161
4	2.311	231.3	4.08993
5	2.844	212.4	2.13403
6	5.727	192.5	0.58255
7	8.859	254.9	1.52953
8	10.509	193.6	0.83845
9	11.459	194.8	0.89837
10	12.059	194.8	0.93643
11	12.554	194.8	0.97463
12	13.625	194.8	1.09007
13	14.125	196	1.15174
14	14.518	196	1.20004
15	15.191	196	1.27047
16	15.858	197.2	1.32678
17	16.891	198.3	1.56607
18	17.608	198.3	1.54604
19	17.907	199.5	1.60032
20	18.357	199.5	1.62626
21	26.970	196	1.13028

Fig. 7 (Cont'd)

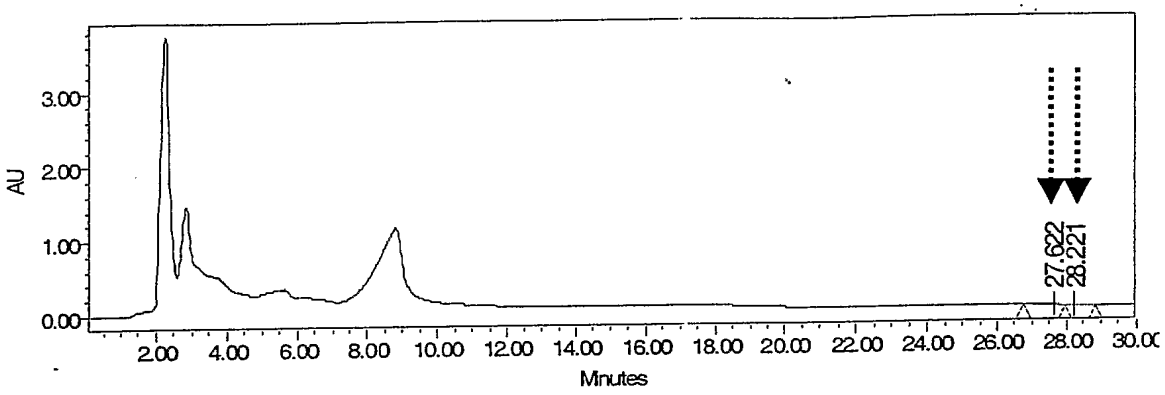
Fig. 7B Tt_Fr_Me_01 at 210 nm



	R. Time	Lambda Max	Abs. Max
1	0.106	658.3	0.00036
2	0.106	658.3	0.00036
3	0.989	203	0.18095
4	1.389	204.2	0.17141
5	2.089	218.3	3.49797
6	2.239	232.4	3.89558
7	2.789	201.9	1.1211
8	3.939	204.2	0.41386
9	5.072	204.2	0.71949
10	5.505	204.2	0.75257
11	7.304	193.6	0.86758
12	8.154	193.6	0.9693
13	9.087	194.8	1.31876
14	10.287	210.1	3.03595
15	11.253	197.2	1.71047
16	**12.692**	**194.8**	**1.22225**
17	13.669	196	1.3187
18	**14.585**	**196**	**1.37959**
19	15.035	196	1.3731
20	15.285	197.2	1.40832
21	15.668	197.2	1.4549
22	16.901	199.5	1.69147
23	17.551	199.5	1.62387
24	18.234	204.2	2.24881
25	18.784	199.5	1.59787
26	19.201	199.5	1.52529
27	19.467	198.3	1.57611
28	21.317	198.3	1.40338
29	**26.832**	**196**	**1.18442**

Fig.7 (Cont'd)

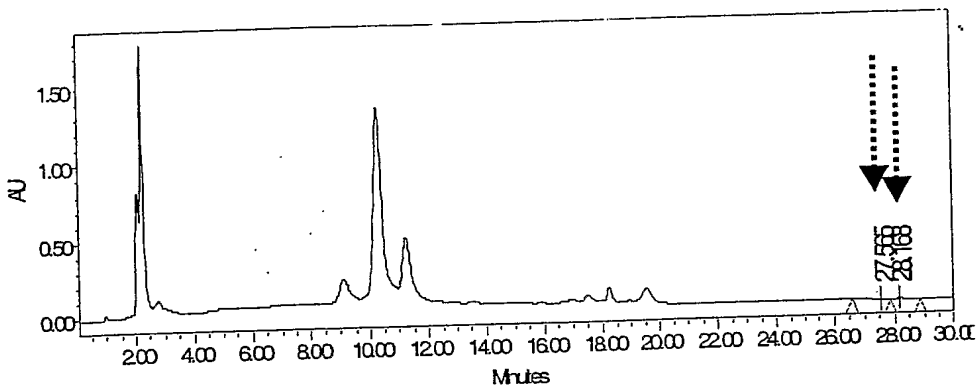
Fig. 7C Ej_Se_Me_01 at 270 nm



	R. Time	Lambda Max	Abs. Max
1	0.111	664.4	0.00017
2	0.928	200.7	0.04692
3	2.278	237.2	4.24142
4	2.844	212.4	2.11013
5	5.643	191.3	0.3677
6	6.26	191.3	0.30824
7	8.809	254.9	1.52352
8	10.526	192.5	0.20265
9	12.842	193.6	0.10449
10	13.408	193.6	0.09651
11	14.158	193.6	0.07521
12	14.642	193.6	0.06469
13	15.158	194.8	0.03509
14	15.525	193.6	0.01212
15	16.358	198.3	0.08989
16	16.558	199.5	0.14244
17	16.924	199.5	0.33248
18	17.757	200.7	0.35602
19	18.207	201.9	0.45986
20	**27.622**	**197.2**	**0.24078**
21	**28.221**	**192.5**	**0.04592**

Fig.7 (Cont'd)

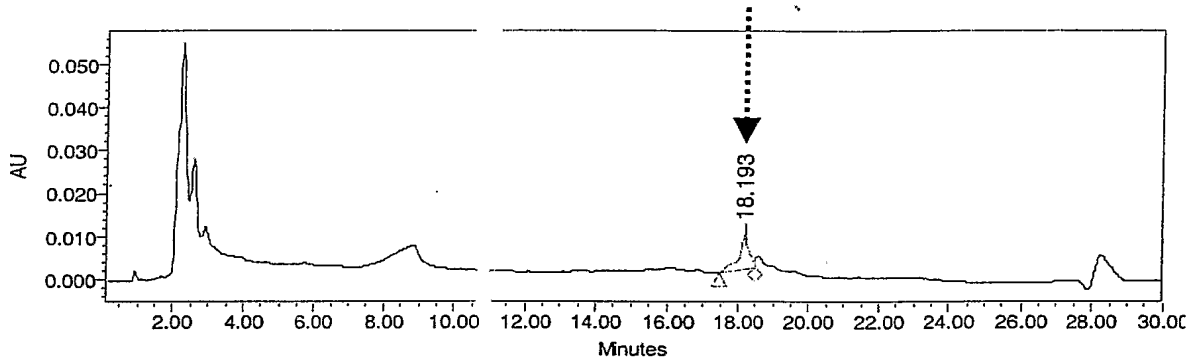
Fig. 7D Tt_Fr_Me_01 at 270 nm



	R. Time	Lambda Max	Abs. Max
1	0.106	658.3	0.00036
2	0.106	658.3	0.00036
3	0.973	204.2	0.17422
4	1.389	206.6	0.15894
5	2.072	218.3	3.49477
6	2.239	232.4	3.89256
7	2.772	203	1.04068
8	3.939	206.6	0.34894
9	5.372	206.6	0.64765
10	6.788	207.7	0.71451
11	7.288	207.7	0.7341
12	7.721	207.7	0.7411
13	8.137	207.7	0.78696
14	8.521	207.7	0.79222
15	9.104	207.7	1.1811
16	10.287	210.1	2.94738
17	11.236	207.7	1.39175
18	12.269	193.6	0.58125
19	12.869	194.8	0.56012
20	13.486	194.8	0.53587
21	13.986	194.8	0.51658
22	14.485	194.8	0.50606
23	14.702	194.8	0.53125
24	15.185	196	0.46804
25	15.469	196	0.44948
26	15.902	196	0.42074
27	16.935	203	0.74559
28	17.468	200.7	0.50841
29	18.234	205.4	1.81022
30	18.634	201.9	0.51603
31	18.917	201.9	0.4448
32	19.534	409.9	0.38359
33	27.565	197.2	0.21209
34	28.165	192.5	0.04792

Fig.7 (Cont'd)

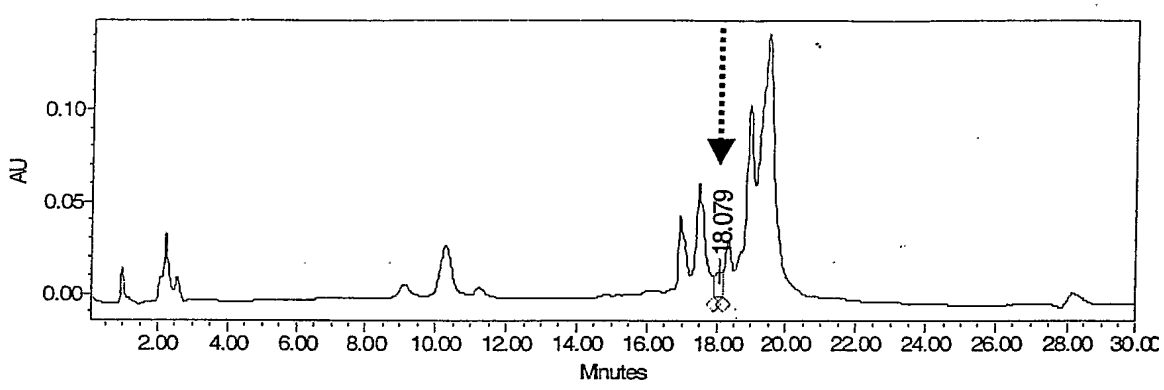
Fig. 7E Ej_Se_Me_01 at 430 nm



	R. Time	Lambda Max	Abs. Max
1	0.111	664.4	0.00017
2	0.928	196	0.0514
3	1.661	223	0.00569
4	2.244	240.7	4.02357
5	2.561	213.6	0.71934
6	2.911	212.4	1.25696
7	8.859	254.9	1.24692
8	**18.193**	**201.9**	**0.20084**
9	18.574	203	0.12088
10	28.239	192.5	0.05227

Fig.7 (Cont'd)

Fig. 7F Tt_Fr_Me_01 at 430 nm



1	0.106	658.3	0.00036
2	0.106	658.3	0.00036
3	0.973	203	0.14779
4	1.789	658.3	0.00616
5	2.239	239.5	3.69315
6	2.572	658.3	0.02939
7	2.905	658.3	0.00356
8	9.104	210.1	0.41005
9	10.287	210.1	2.28091
10	11.236	204.2	0.80513
11	13.186	191.3	0.00125
12	14.835	407.5	0.00123
13	16.152	437.7	0.00304
14	16.935	201.9	0.60222
15	17.451	200.7	0.38
16	**18.079**	**201.9**	**0.44026**
17	18.301	204.2	1.26276
18	18.667	201.9	0.39938
19	18.917	201.9	0.34766
20	19.451	409.9	0.45206
21	28.165	192.5	0.04642

Fig 8: Avestha's *Metagrid* to achieve relevant therapeutic extracts/molecules.

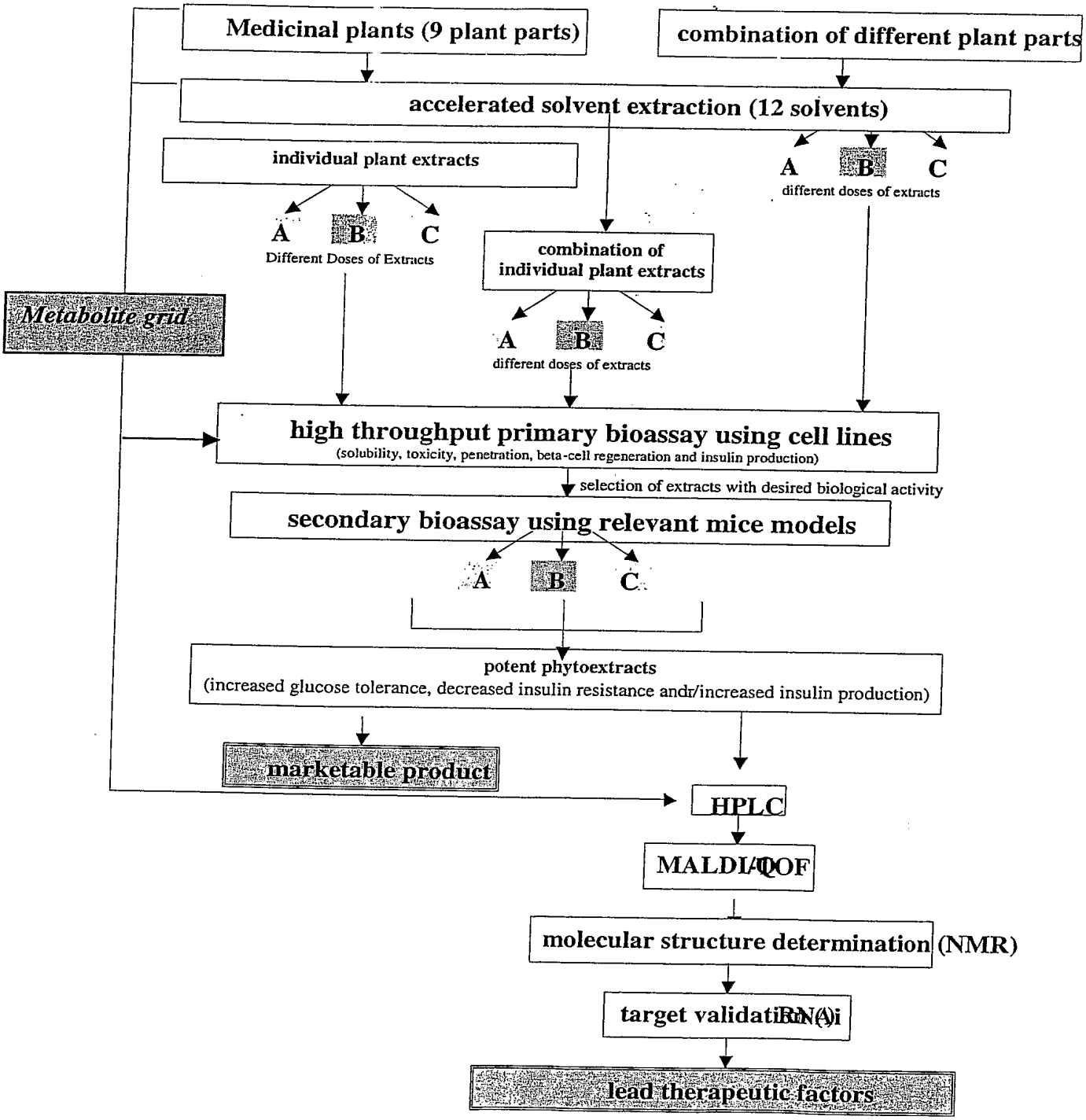


Fig 9: Extraction process used to arrive at the *Metagrid*

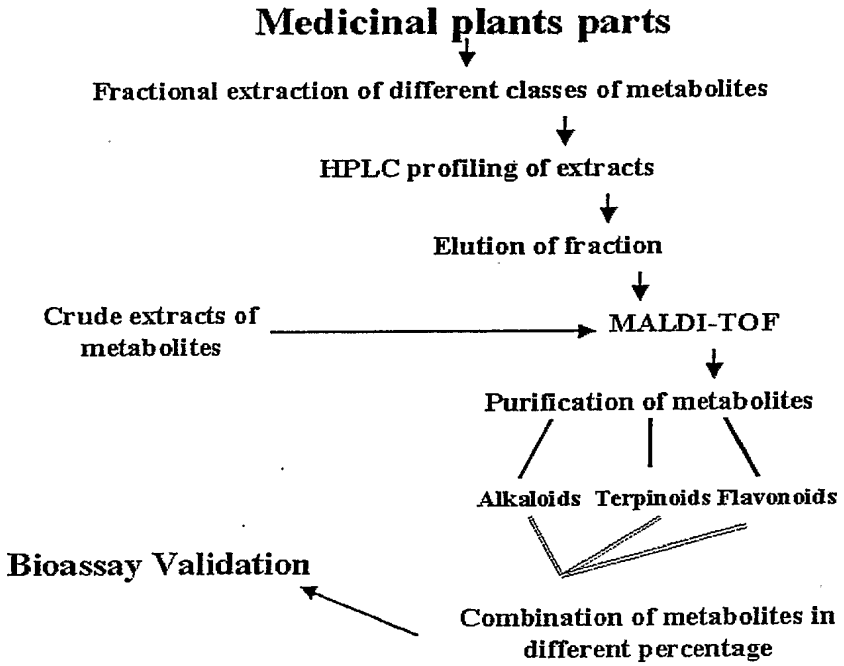


Fig 10: Successive Extraction Process

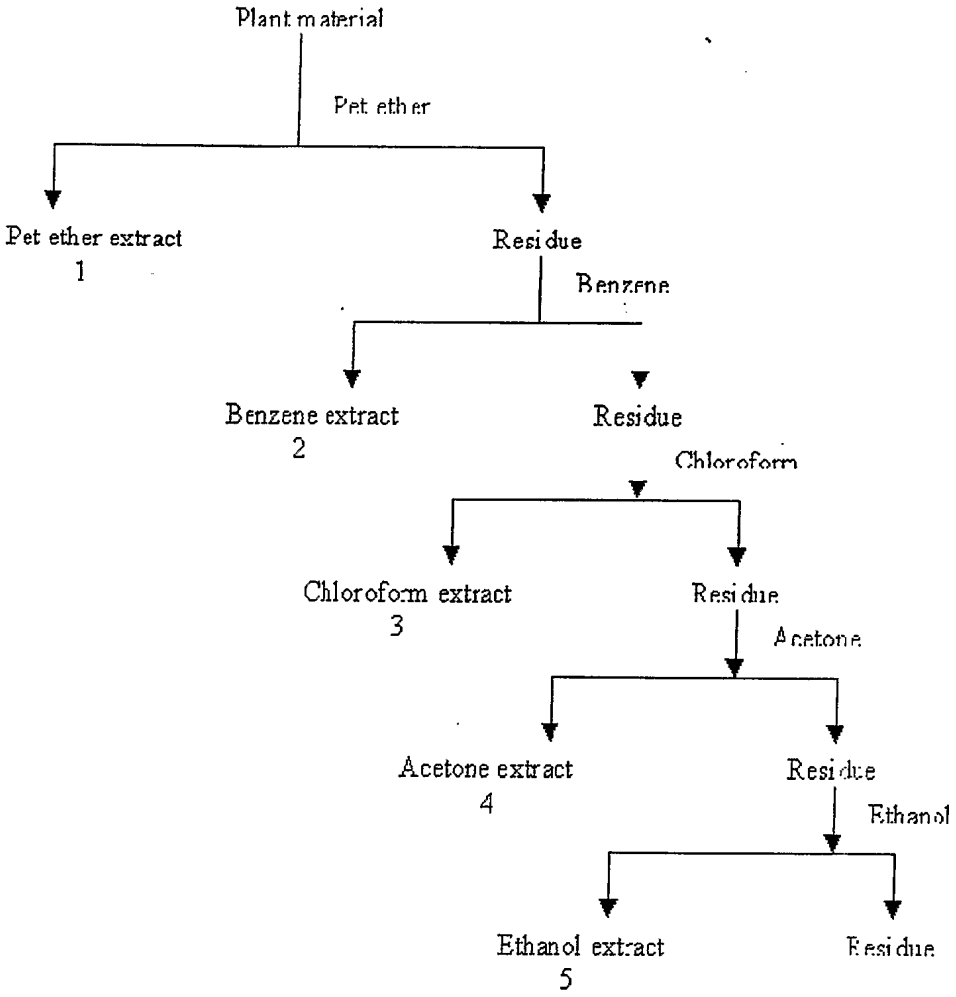
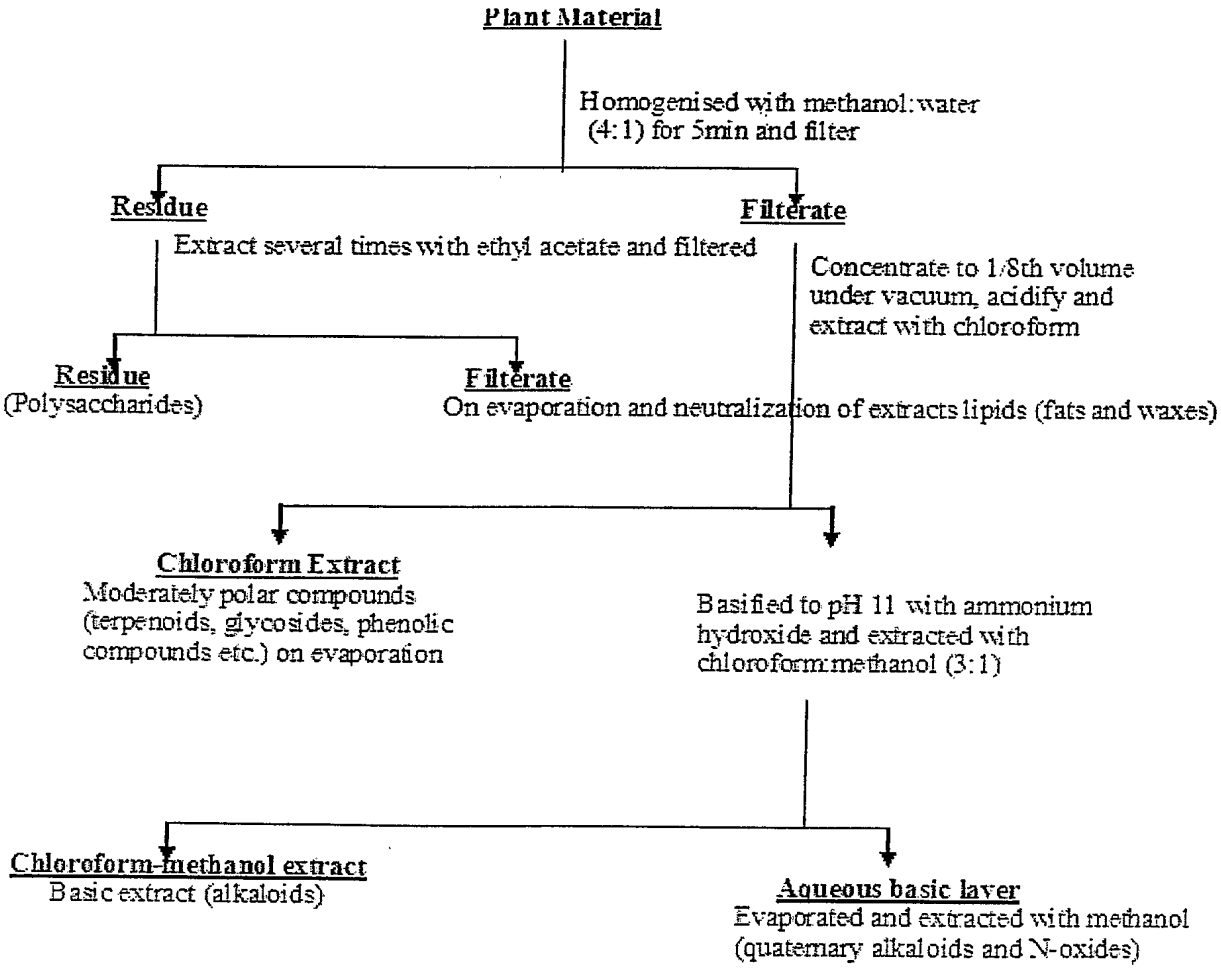


Fig 11: Fractional Extraction Procedure



Novel therapeutic extracts and molecules (Yezdex) for Diabetes using Avestha's metabolite grid (*Metagrid*)

Field of the Invention

The present invention relates to a plethora of selected plants, which were isolated and characterized for the therapeutically relevant molecules to be used in the treatment of Diabetes. The extracts from the plants were subjected to both, targeted and non-targeted screening procedures. The ongoing-targeted screening procedures, which feature a comprehensive metabolite profiling of multitudes of phyto-extracts, were envisaged to facilitate the creation of a metabolite grid. Extensive comparative analyses of the individual plant species with the existing drug and/or phyto-extract formulations in the market has revealed the presence of both, unique and common molecular constituents that will be used individually and/or in combination to accelerate the process of the discovery of novel therapeutic formulations. This invention also relates to an edible composition comprising of fifteen Indian herbal extracts which can be used as a dietary supplement and also useful in lowering the glucose levels in the blood of mammals, particularly humans, suffering from Diabetes *mellitus*.

Background of the Invention

Diabetes is often defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and post-prandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to a condition known as hyperglycemia that will in due course turn into a syndrome called Diabetes *mellitus*. The word Diabetes is derived from [illegible] Greek work "Diab", meaning to pass through, namely, refer[illegible]t urination. *Mellitus* is derived from the Latin word "sweete[illegible] of sugar in the urine. Diabetes *mellitus* is the most common [illegible]etabolic disorder characterized by a lack of hormone insu[illegible]alities in the assimilation of carbohydrates in the body. Di[illegible]f syndromes characterized by hyperglycemia, altered metab[illegible]long with increasing risks of complication from vascular [illegible]e disease is realized on the development of secondary sy[illegible]ely, difficulty in healing of wounds, neuropathy and so on[illegible]ion people all over the world suffer from Diabetes *mellitus* a[illegible]n Diabetes; it is not properly diagnosed until irreversible con[illegible]

pages replaced

Diabetes has been known to [illegible]enturies and early recorded literature has even documented [illegible]betes around 1,000 B.C. and later on the physician, Charak[illegible]: the syndrome of Diabetes *mellitus*, existed in India since [illegible] earliest reference (1,000 B.C.) in the Ayurvedic literature is found in mythological form, where it is said to have originated by eating Havisha, a special

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Diabetes has been known to be prevalent in India since the past few centuries and early recorded literature has even documented the physician, Sushruta's description of Diabetes around 1,000 B.C. and later on the physician, Charak. It is in fact believed that the knowledge of the syndrome of Diabetes *mellitus*, existed in India since pre-historic age. Its earliest reference (1,000 B.C.) in the Ayurvedic literature is found in mythological form, where it is said to have originated by eating Havisha, a special

food that used to be offered at the time of Yagna organised by Daksha Prajapati (Kohli et al., Ayurvedic perspective of Diabetes and allied disorders, Vedic Life sciences).

However in developing countries like India, there is not much focus on Diabetes, given its chronic and slow nature. Moreover the economics of developing countries do not permit for devoting its resources to an understanding or search for remedies. The World Health Organisation has projected India, as the country with the fastest growing population of diabetics and it is estimated that between 1995-2025, Diabetes patients in India will increase by 195%.

Amongst the many classical, clinical symptoms associated with Diabetes, one which is typical, is an increase in the blood glucose, otherwise known as hyperglycemia, which in turn may result in polyuria (frequent urination), polydipsia (excessive thirst), polyphagia (excessive hunger), weight loss and blurred vision, apart from glycosuria and acetone breath. The long terms complications arising out of untreated or ineffectively treated Diabetes include among others, retinopathy, nephropathy and peripheral neuropathy. Diabetes patients stand an increased risk of succumbing to cardiovascular diseases and strokes.

Recent developments in understanding the pathophysiology of the disease process have opened up several new avenues to identify and develop novel therapies to combat the diabetic plague. Phytochemicals identified from traditional medicinal plants present an exciting opportunity to develop new kinds of therapeutics. There is an urgent need to identify indigenous natural resources, procure them and study them in detail and their potential on newly identified targets in order to develop them as new therapeutics.

The increasing cost of modern treatment of Diabetes indicates a great need for the development of alternate strategies for the prevention and treatment of Diabetes. In rural pockets of developing countries, almost 90% of the population still rely on traditional medicines for their primary health care and an investigation into such traditional medicines have led to the discovery of at least 88 drugs. There is a need for a rationally designed interdisciplinary research programme, which could lead to the development of indigenous, renewable medicinal plant sources as practical and cost effective alternatives. It is believed that the therapeutic approach of several traditional medicinal systems is more holistic. The medicinal preparations from traditional medicines contain a variety of herbal and non-herbal ingredients that are believed to act on a variety of targets through various modes and mechanisms.

Since ancient times, traditional medicines all over the world have advocated the use of plants to treat Diabetes. The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of beta cells, insulin releasing activity, improvement of the glucose uptake, utilisation and the anti oxidant properties present in the medicinal plants, which offers exciting opportunities, to develop them into novel therapeutics.

The multifactorial pathogenicity of Diabetes demands a multi – modal therapeutic approach. Thus, future therapeutic strategies require the combination of various types of multiple agents. *Medicatrix naturae* or the power of self-preservation or adjustment has been the motto of traditional medicinal

practise, which prescribes poly herbal formulations. The theories of poly herbal formulation have the synergistic, potentiative, agnoistic/antognistic pharmacological agents within themselves due to the incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles are known to work in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. Traditional medicines ought not to be treated as a collection of therapeutic recipes. They are formulated and prepared, keeping in mind, the disease/sickness and the healing properties of the individual ingredients.

In the case of Diabetes, the main symptoms targeted were thirst, polyuria and glycosuria (Herbal Drugs as Antidiabetics: An Overview; Sanjay M. Jachak, CRIPS Vol. 3, Number 2, April-June 2002). There have been over 1200 plants that have been screened for activity on the basis of ethnopharmacology or on a random basis. Plant extracts have been tested under specific conditions as glucose-loaded, alloxan, streptozotocin or naturally diabetic subjects in various animals like rodents.

The Indian subcontinent is renown for its indigenous collection of natural remedies like Ayurveda, Unani, Siddha and so on, based on which, new therapeutic molecules can be obtained. A large number of drugs from plant sources are secondary metabolites, which have no role in plant metabolism, but are expected to play a significant role in plant defence mechanism. There is however, not much difference seen between the basic metabolic processes in plants and animals. There are approximately around 200 pure compounds from plant sources, which are reported to indicate a decrease in the blood glucose.

Over 1200 plant compounds worldwide have undergone tests at various levels in order to ascertain their ability to lower blood sugar levels and it was found that many of these compounds contained chemical components that possessed hypoglycemic activity, when, tested in either animal models or test tubes. However the research on such compounds involving human subjects has not brought much information to light so far. There have been a few herbal remedies for Diabetes tested on humans and these have revealed mild blood sugar lowering properties.

Type II Diabetes also known as non-insulin dependent Diabetes *mellitus* ('NIDDM') is more common than insulin dependent Diabetes *mellitus*, affecting 80-90% of all people affected with Diabetes. Initially NIDDM is often of gradual onset during the latter middle age. Later stages of this disease are very severe resulting in long term complications like kidney failure, heart problems, eye and nervous disease and other diseases as well. Obesity is an important factor in NIDDM. The symptoms can be vague and include fatigue, nausea, frequent urination and unusual thirst. NIDDM develops genetically in predisposed individuals. The pathological changes in the pancreatic islets of patients with NIDDM are not always apparent. Many patients are known to have normal to high plasma insulin levels. In such cases, very often, Diabetes does not arise from a shortage of insulin, but as a result of defects in the molecular machinery that mediates the action of insulin on its target cells. NIDDM is also caused by destruction of other mechanisms such as insulin resistance, down-regulation of insulin receptors, defects in insulin secretion from the pancreatic cells and other changes to the glucose transporter system. To date, no satisfactory method exists to treat or cure NIDDM without apparent toxicity to the patients. Therefore there is an urgent need to provide alternative treatments, effective in the prevention and/or treatment of NIDDM.

An ideal medication for the treatment and prevention of Diabetes would be one which would incorporate the following characteristics: ability to stimulate regeneration of pancreatic islets and beta cells responsible for insulin production and to increase c-peptide levels; ability to modulate the autoimmune destruction of the cells responsible for insulin production; ability to correct the dislipidemia associated with Diabetes; ability to decrease insulin resistance, with few or no side effects. However the known pharmaceutical compositions for treating Diabetes do not meet with this criterion. The pharmaceutical oral hypoglycemic agents produce inconsistent clinical results with frequent, severe side effects and there is a need felt for safe and effective oral hypoglycemic agents that provide the clinician with a wide range of options to prevent, treat and manage Diabetes since it has been held that the traditional drugs prescribed to balance blood sugar can result in serious liver damage and in some cases, even cause liver failure. Although many traditional plant treatments for Diabetes exist, it is only a few that have received scientific or medical scrutiny (Gray & Flatt Insulin-secreting activity of the traditional anti diabetic plant Viscum album (mistletoe) – Journal of Endocrinology, 1999).

Herbs have been frequently alluded to as part of 'nature's pharmacy'. Even though herbal drugs act in more than one way analogous to modern drugs, herbal remedies are generally looked upon as a lot safer. In fact, many of the drugs used in conventional medicine are derived from herbs, instead of isolating the 'active agent'. Herbalism uses the whole plant. In many cases it has been observed that plants contain constituents that work together synergistically and using the whole plant aids in decreasing the side effects that may occur when using isolated components.

Plants with medicinal properties, the gift of Mother Nature to mankind, have been in use in India over several centuries in the traditional systems of medicine like Ayurveda, Unani etc., for the treatment of diseases including Diabetes *mellitus*. They are considered to be effective and toxic. It is botanical medicine that provides a complete system of healing and prevention of the disease. It is the oldest and most natural form of medicine. Its history of efficacy and safety spans centuries and covers almost the entire planet. Since herbal medicine is holistic medicine, it is able to look beyond the symptoms to the underlying systemic imbalance and offers real and permanent solutions to the problems on hand.

Higher plants constitute one of our most important natural resources. They not only provide foodstuffs, fibers, and woods, but many biochemicals, such as oils, flavorings, dyes, and pharmaceuticals. Although plants are renewable resources, some species are becoming more difficult to obtain in sufficient amounts to meet increasing demands. Destruction of natural habitats and technical difficulties in cultivation too are responsible for the drastic reduction in plant availability. For example, it is claimed that a demand for paclitaxel, a potent anticancer compound, could endanger the forests of *Taxus brevifolia* (Pacific yew) because of the low paclitaxel content (40–100 mg/kg of bark) in and a slow growth of the trees.

For many natural chemicals it is possible to synthesize alternatives from petroleum, coal, or both. The economic limitations of chemical synthesis and the pollution that accompanies this type of chemical synthesis, however, have led to the development of cell culture and molecular engineering of plants for the production of important chemicals. Plant cell and organ cultures offer promising alternatives for the production of biochemicals since totipotency enables plant cells and organs to produce useful secondary metabolites *in vitro*. Molecular engineering of secondary metabolites has the potential to increase productivity and improve product composition.

The metabolism comprises a coordinate series of coupled enzymatic conversion in living organisms. The secondary metabolites are not vital to the cell death that produces them but contribute to the overall fitness of the organisms. The functions of these compounds in plants include protection against pests and pathogens. For man, plant secondary metabolites are useful as pharmaceutical dyes, fragrance, insecticides and/or flavours.

In order to regulate the biosynthesis of secondary metabolites, plants must accommodate their primary metabolic pathways. A coordinate regulation between these processes has been observed but the regulatory mechanisms are unknown. (Lelslie van der Fits & Johan Memelink, 2000). Production of secondary metabolites is controlled at the levels of expression of the biosynthetic genes by developmental tissue specific factors or by external signals. The accumulation of metabolites is induced by (methyl) jasmonate, a plant hormone produced in response to stress.

A biosynthesis of many classes of secondary metabolites in plants is induced by the stress hormone, jasmonate. The gene for ORCA-3, a jasmonate responsive APETALA2 (AP2) domain transcription factor was isolated by transferred DNA activation tagging and its over expression resulted in an enhanced expression of several metabolites biosynthetic genes and consequently, an increased accumulation of terpenoid Indole alkaloids. A regulation of metabolites biosynthetic genes by jasmonate responsive AP-2 domain transcription factors may link plant stress responses to changing metabolism. Plants can regulate primary metabolic pathways coordinately with secondary metabolism using a single transcription factor. Since the biosynthesis of many secondary metabolites is induced by jasmonate, the identification of an AP2 domain protein as a regulator of several genes involved in JA responsive metabolism uncovers a control mechanism that may be operative in other stress responsive plant metabolic pathways as well.

Prior Art

US Patent number 5980902 states that the leaves of *Gymnema sylvestre*, a herb belonging to the Asclepiadaecae family have been used by traditional medical practitioners of India to treat diabetic conditions for several centuries. *Gymnema sylvestre* has also been studied for its anti sweet properties, for its ability to inhibit small intestine absorption of glucose and for its ability to suppress increases in blood glucose levels following glucose administration. US Patent number 5900240 refers to *Syzgium cumini* jamun, *Gymnema sylvestre, Momordica charantia* bitter gourd and *Solanum melongena* egg plant, the compositions of which will provide a herbal dietary supplement, which will be tolerated by insulin dependent diabetic sufferers without any undesirable side effects and which will allow blood glucose levels to be controlled to a level below that achievable by administration of insulin. European Patent number WO9842211 has claimed for nutritional supplements, which could be used as a treatment for poor glucose metabolism of Diabetes, and also for prevention of Diabetes by giving the metabolism a boost before the full-blown Diabetes develops. The purpose of Patent number JP4022627 has been to obtain an insulin secretagogue, useful as a curing agent for a patient suffering from hyperglycemia, ketoacidosis etc. instead of insulin.

CN1380072 patent states a medicine for preventing and curing diabetes, other metabolic disease and its complicating diseases. It includes extract of dried or fresh plant *Gymnema sylvestre* and voglepotang sugar, as compared with existent technology it has the advantages of high therapeutic effect, low toxic side effect and long acting time, not only can be used for preventing and curing various diabetes, but also can be used for preventing and curing hyperlipemia, adiposis, arteriosclerosis, X syndrome and other complicating diseases.

US patent 5980902 describes compositions derived from *Gymnema sylvestre* leaf materials that may be administered orally, intravenously, subcutaneously or transdermally which are useful for treating patients having diabetes, impaired glucose tolerance, and various conditions associated with or symptoms of diabetes. Additionally, the compositions reduce polydipsia, polyuria and polyphagia, regenerate the pancreatic islets of Langerhans, including beta cells, increase endogenous insulin, lipase and amylase levels, increase production of proinsulin and c-peptide, and lower blood lipids and triglycerides and free fatty acids.

European patent number WO9510292 talks about glucose metabolism in a human patient being regulated by dosage forms that contain a naturally occurring, plant derived carbohydrate, an aqueous and water-miscible polar solvent extract, preferably an aqueous and ethanolic extract, of *Gymnema sylvestre* in combination with a non-metabolizable polysaccharide preferably a Sterculia urens exudate, in a respective weight ratio in the range of about 1:2 to about 2:1.

Aqueous extracts from the leaves of *G. sylvestre* have been described as inhibiting temporarily the taste of sweet substances. It has also been reported that the raw leaves of *G. sylvestre* have been used in India as a folk medicine for various afflictions including diabetes mellitus. Some fourteen or fifteen different compounds are reported to have been isolated from the leaves of *G. sylvestre* by various techniques. Stocklin, J. Agr. Food Chem. 17(4):704-708 (1969); Sinsheimer, J. Pharm. Sci. 59(5):622-628 (1970). However, applicant is not aware of scientific information as to whether any of the noted chemicals, individually or collectively, contribute to the hypoglycemic properties. It has now been found, however, that the present aqueous and water-miscible polar solvent extract contains at least four fractions that are insulinotropic. Two of these fractions exhibit substantially equal and relatively strong insulinotropic activity.

Chinese patent CN1268515 relates to the extract of *Gymnema sylvestre* which is mainly composed of total triterpene saponin, flavone glycoside, anthocyan,polysaccaride, etc. in which the content of total triterpene saponin is 50-99%, and 25-40% of the total triterpene saponin are six kinds of new triterpene saponin compounds. Said extract possesses the active functions of lowering blood sugar, bloodfat and anti-thrombocyte coagulation.

US patent 6,572,897 describes a composition that contains essential amounts of Alpha Lipoic Acid, Chromium, Lutein, Bioflavonoids(quercetin and rutin), Mormordica Charantia extract, Corosolic Acid, and *Gymnema Sylvestre* Extract, as well as other ingredients and healthy filler ingredients with clinical studies proven to assist in the maintenance of insulin sensitivity and healthy blood sugar levels.

US patent 5,886,029 describes a medicinal composition including a pharmacologically significant quantity of (-)epicatechin augmented with a comparable amount of gymnemic acid useful for the treatment of diabetes in a human subject. The medicinal composition of the invention induces a significant reduction in serum glucose due to the regeneration of pancreatic islet cells. The unique combination of components in the medicinal composition leads to a regeneration of the pancreas cells, which then start producing insulin on their own. Since the composition restores normal pancreatic function, treatment can be discontinued after between about four and twelve months.

US patent 5,137,921 describes the use of an inhibitory agent of an increase in blood sugar level, conduritol A obtained from dried leaves of *Gymnema sylvestre* or from dried bark of Marsdenia condurango by means of extraction.

Shamnugasundaram et al studied the use of *Gymnema Sylvestre* leaf extract in the control of blood glucose in Insulin-dependent Diabetes Mellitus, Journal of Ethnopharmacology 30,pp.281-294,1990.

Baskaran et al studied the antidiabetic effect of a leaf extract from *Gymnema Sylvestre* in Non-insulin-dependent Diabetes Mellitus, Journal of Ethnopharmacology 30, pp. 295-305, 1990. Patients were able to discontinue their conventional drug and maintain their blood glucose homeostasis with the extract of *Gymnema Sylvestre*(GS4) alone. These data suggested that the beta cells might be regenerated/repaired in Type 2 diabetic patients on GS4 supplementation. This is supported by the appearance of raised insulin levels in the serum of patients after GS4 supplementation.

Sugihara Y et al. described the antihyperglycemic effects of gymnemic acid IV, a compound derived from *Gymnema sylvestre* leaves in streptozotocin-diabetic mice, J Asian Nat Prod Res. 2000;2 (4):321-7. Gymnemic acids derived from the methanol extract of leaves of Gymnema sylvestre, at doses of 3.4-13.4mg/kg reduced the blood glucose levels by 13.5-60.0% 6h after the administration, comparable to the potency of glibenclamide. These results indicate that insulin-releasing action of gymnemic acid IV may contribute to the antihyperglycemic effect by the leaves of G. sylvestre. Gymnemic acid IV may be an anti-obese and antihyperglycemic pro-drug.

Rathi et al. studied the effect of *Gymnema sylvestre* on protein-bound polysaccharide components & glycosaminoglycans in experimental diabetes. Indian J. Experimental Biol 19, pp. 715-721, 1981.

Shanmugasundaram et al. tried to find the possible effects of leaf extracts of *Gymnema Sylvestre* on the regeneration of the islets of langerhans in Streptozotocin-diabetic Rats. Journal of Ethnopharmacology 30, pp. 265-279, 1990.

CN1122699 describes capsules made from balsom pear, lagenaria peel, root of Chinese angelica, periwinkle etc. The clinical tests on more than 1000 cases of diabetes patients who do not rely upon insulin therapy verified that the total effective rate is up to 92.5%. It also has notable effect for reducing the blood sugar for diabetes patients relying upon insulin.

Singh SN et al. studied the effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats, J Ethnopharmacol. 2001 Aug;76(3):269-77. Extract at dose 500 mg/kg given orally for 7 and 15 days showed 48.6 and 57.6% hypoglycemic activity, respectively. Prior treatment at the same dose for 30 days provided complete protection against STZ challenge (75 mg/kg/i.p.x1). Results indicate increased metabolization of glucose in treated rats. Increased levels of lipid peroxidation measured as 2-thiobarbituric acid reactive substances (TBARS) indicative of oxidative stress in diabetic rats were also normalized by treatment with the extract.

United States Patent Application 20010021401 relates to an herbal therapeutic product for controlling diabetes mellitus comprising at least one hypoglycemic compound extracted from the pulp of a fruit from a species of genus Eugenia specifically *Eugenia jambolina* and a process for the preparation of the same.

US patent application 20020025349 describes a synergistic oral liquid herbal composition falling under the category of "Asavas" and "Arishtas", useful for management of diabetes, said composition comprising a therapeutically effective amount of plant extracts selected from a. *Momordica charantia* (2-5%), b. *Gymenma sylvestre* (8-12%), c. *Pterocarpus marsupium* (8-12%), d. *Eugenia jambolana* (4-10%), and e. *Trigonella foenum grecum* (1-3%), and, optionally, comprising extracts/powder of *Woodfordia fruticosa* (2 to 5%), *Piper longum* (0.1 to 0.3%), *Elettaria cardamomum* (0.1 to 0.3%), *Myristica fragrans* (0.1 to 0.3%) and *Ammomum subulatum* (0.1 to 0.3%).

US patent 5,972,342 describes mixtures isolated from grains of *Eugenia Jambolana Lamarck*, the preparation of such mixtures, the medicaments containing said mixtures or constituents of said mixtures, and the use of these mixtures for the treatment of diabetes and complications associated with Diabetes.

Kelkar in his article XP-000940531, Phytomedicine Volume 3 (4) pages 353-359,1996/97, described a simple two step purification of antidiabetic compounds from *Eugenia jambolana* fruit-pulp; proteolytic resistance and other properties.

Rathi SS et al., assessed the efficacy of *Momordica charantia* (MC), *Eugenia jambolana* (EJ), *Tinospora cordifolia* (TC) and *Mucuna pruriens* (MP) in the prevention of murine alloxan dibetic cataract. (Phytother Res. 2002 Dec;16(8):774-7). The incidence rate of cataract in MC, EJ, TC and MP treated groups at 120 days was only 0, 0, 1 and 2. Oral feeding of MC, EJ, TC and MP extracts for 1 month produced a fall of 64.33%, 55.62%, 38.01% and 40.17%, respectively, in the serum glucose levels in comparison with the 48 h level. After 2 months of treatment, the respective values were 66.96%, 59.85%, 40.41% and 45.63%. MC and EJ prevented the development of cataract while the protective effect was less with TC and MP along with a significant reduction of plasma glucose levels.

Grover JK et al., studied the amelioration of experimental diabetic neuropathy and gastropathy in rats following oral administration of plant (*Eugenia jambolana, Mucuna pruriens and Tinospora cordifolia*) extracts, Indian J Exp Biol. 2002 Mar;40(3):273-6.

Anila L et al. studied the beneficial effects of flavonoids from *Sesamum indicum, Emblica officinalis* and *Momordica charantia*. Of the three sources, flavonoids isolated from *Emblica officinalis* exerted the

maximum beneficial action by eliciting highly potent hypolipidaemic and hypoglycemic activities. (Phytother Res. 2000 Dec;14(8):592-5)

Vikrant et al. tried to study the efficacy of the extracts of *Momordica charantia* and *Eugenia jambolana* to prevents hyperglycemia and hyperinsulinemia in fructose fed rats, J Ethnopharmacol. 2001 Jul;76(2):139-43.

Sharma SB et al. studied the hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan-induced diabetic rabbits. J Ethnopharmacol. 2003 Apr;85(2-3):201-6.

US patent 6,551,627 15 describes an herbal medicinal composition for preventing or treating type II diabetes. The composition is comprised of extracts from *Pterocarpus marsupium, Morus alba, Orthosiphon aristatus, Opiophogon japonicus, Rosa rugosa, Commelina communis, Trichosanthis kirilowii* and *Anemarrhena asphodeloides*. The patent also describes the effect of the composition in reducing blood glucose level in a patient who has blood glucose level of about 200 to about 300 mg/dl at the beginning of treatment, increasing insulin secretion from pancreatic beta cells and a method of inhibiting degradation of complex carbohydrates to monosaccharides.

US patent 6,448,450 talks about diphenylethylene *Pterocarpus marsupium* which when administered orally decreases blood glucose levels in rats. The compound is an effective anti-diabetic agent that can reduce abnormality of glucose metabolism in diabetes.

European patent application WO0172316 speaks about an edible Ayurvedic herbal composition for reducing blood sugar levels in humans, specially suffering from diabetes mellitus comprising a mixture of ingredients selected from the group consisting of *Cinnamomum zeylanicum, Artocarpus heterophyllus, Salacia reticulata, Tinospora cordifolia* and *Pterocarpus marsupium*. The mixture of the ingredients of the five selected herbs present in therapeutically effective proportions depending on the required strength of the mixture to treat abnormal levels of blood sugar and diabetes mellitus.

US patent 6,562,791 describes a novel glucopyranoside, 6-hydroxy-2-p hydroxybenzylbenzofuran-7-C-.beta.-D-glucopyranoside isolated from *Pterocarpus marsupium* and to a process for the isolation thereof. The invention also relates to a pharmaceutical composition containing 6-hydroxy-2-p-hydroxybenzylbenzofuran-7-C-.beta.-D-glucopyranoside and to method for the treatment of diabetes using said compound.

Manickam M et al. studied the antihyperglycemic activity of phenolics from *Pterocarpus marsupium*, J Nat Prod. 1997 Jun;60(6):609-10. Of the 3 important phenolic constituents of the heartwood of Pterocarpus marsupium, marsupsin (1), pterosupin (2), and pterostilbene (3), Marsupsin and pterostilbene significantly lowered the blood glucose level of hyperglycemic rats, and the effect was comparable to that of 1,1-dimethylbiguanide (metformin).

Sheehan EW discovered a constituent of *Pterocarpus marsupium*, (-)-epicatechin, as a potential antidiabetic agent. J Nat Prod. 1983 Mar-Apr;46(2):232-4. (-)-Epicatechin was found to reverse hyperglycemia in alloxan diabetic rats when given before or within 24 hr after the dose of alloxan.

However, when doses of (-)-epicatechin (30 mg/kg, i.p., twice daily for 3 days) are begun 92 hr after alloxan, there is no significant difference in blood glucose levels between control and (-)-epicatechin treated rats.

Gupta SS et al. studied the effect of *Tinospora cardifolia* on fasting blood sugar level, glucose tolerance and adrenaline induced hyperglycaemia. Indian J Med Res. 1967Jul;55(7):733-45.

Prince PS et al. studied the antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. J Ethnopharmacol. 1999 Jun;65(3):277-81. *T. cordifolia* root extract (TCREt) (2.5 and 5.0 g/kg) for 6 weeks resulted in a decrease in the levels of plasma thiobarbituric acid reactive substances, ceruloplasmin and alpha-tocopherol in alloxan diabetic rats. The root extract also causes an increase in the levels of glutathione and vitamin C in alloxan diabetes. The root extract at a dose of 5.0 g/kg showed the highest effect. The effect of TCREt was more effective than glibenclamide.

Stanely P et al. studied the hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats, J Ethnopharmacol. 2000 Apr;70(1):9-15. Oral administration of an aqueous T. cordifolia root extract (TCREt) to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids. The extract caused an increase in body weight, total haemoglobin and hepatic hexokinase. The root extract also lowers hepatic glucose-6-phosphatase and serum acid phosphatase, alkaline phosphatase, and lactate dehydrogenase in diabetic rats.

Li M et al. studied the hypoglycemic effect of saponin from *Tribulus terrestris*, Zhong Yao Cai. 2002 Jun; 25(6): 420-2. The level of serum glucose could be significantly reduced by saponin from *Tribulus terrestris*, which was the rate of 26.25% and 40.67% in normal mice and diabetic mice in respectively. The level of serum triglyceride could be reduced 23.35%.

US patent 6,042,834 describes a herbal composition for the treatment of diabetes, comprising 15 percent by weight of dried, powdered seeds of *Trigonella foenum-graecum*; 23 percent by weight of dried, powdered seeds of *Nigella sativa*; 10 percent by weight of dried, powdered leaves of *Origanum vulgare*; 10 percent by weight of dried, powdered sap of *Rosmarinus officinalis*; 15 percent by weight of dried, powdered beans of *Lupinus termis*; 12 percent by weight of dried, powdered black leaves of Lawsonia inermis; and 15 percent by weight of dried, powdered seeds of *Foeniculum vulgare*.

An Indian patent application 305/MAS/99 describes a process for preparation of an antidiabetic herbal drug from the plants *trichopus zeylanicus, withania somnifera* and *piper longum*.

Andallu B et al studied the hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera*, Dunal) root, Indian J Exp Biol. 2000 Jun;38(6):607-9. Hypoglycemic, diuretic and hypocholesterolemic effects of roots of *W. somnifera* (ashvagandha) were assessed on human subjects. Decrease in blood glucose was comparable to that of an oral hypoglycemic drug. Significant increase in urine sodium, urine volume, significant decrease in serum cholesterol, triglycerides, LDL (low density lipoproteins) and VLDL (very low density lipoproteins) cholesterol were observed indicating that root of *W. somnifera* is a potential source of hypoglycemic, diuretic and hypocholesterolemic agents.

US patent 5,470,879 describes a process for stimulating the secretion of insulin and for the treatment of non-insulin dependent diabetes by the administration of effective quantities of substantially pure 4-hydroxyisoleucine or its lactone form or mixtures thereof obtained from *Trigonella foenum graecum L.*

Vats V et al. evaluated the anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats, J Ethnopharmacol. 2002 Jan;79(1):95-100.

Abdel-Barry JA et al. studied the hypoglycemic effect of aqueous extract of the leaves of *Trigonella foenum-graecum* in healthy volunteers, East Mediterr Health J. 2000 Jan;6(1):83-8.

Ali L et al. characterized the hypoglycemic effects of *Trigonella foenum graecum* seed, Planta Med. 1995 Aug; 61(4):358-60. The whole powder of *Trigonella foenum graecum* seeds and its extracts were tested for their hypoglycemic effect on normal and diabetic model rats. The powder, its methanol extract, and the residue remaining after methanol extraction had significant hypoglycemic effects when fed simultaneously with glucose.

Zia T et al. evaluated the oral hypoglycaemic effect of *Trigonella foenum-graecum* L. (methi) in normal mice, J Ethnopharmacol. 2001 May;75(2-3):191-5. The presence of hypoglycemic activity in aqueous and methanolic extract indicates that the active compounds are polar in nature.

Gupta A et al. studied the effect of *Trigonella foenum graecum* (fenugreek) seeds in glycemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. J Assoc Physicians India. 2001 Nov; 49:1057-61. Adjunct use of fenugreek seeds improves glycemic control and decreases insulin resistance in mild type-2 diabetic patients. There is also a favorable effect on hypertriglyceridemia.

Sharma RD et al. studied the effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes, Eur J Clin Nutr. 1990 Apr;44(4):301-6. The fenugreek diet significantly reduced fasting blood sugar and improved the glucose tolerance test. There was a 54 per cent reduction in 24-h urinary glucose excretion. Serum total cholesterol, LDL and VLDL cholesterol and triglycerides were also significantly reduced. The HDL cholesterol fraction, however, remained unchanged. These results indicate the usefulness of fenugreek seeds in the management of diabetes.

In US Patent number 6391854, a water-soluble fraction of *Momordica charantia* and methods for its preparation and use in the treatment of hyperglycaemic disorders, is provided for. In Patent number RO116044 patent application, the process of extracting the immature, dried, milled fruit of *Momordica charantia* with methanol and concentrating it in an ethanolic solution till a viscous product is obtained, is a process for preparing a medicine for internal use and used as an adjuvant in the therapy of Diabetes *mellitus*. In US Patent number 6127338, a water-soluble extract of *Momordica charantia*, methods for its preparation and use in the treatment of hyperglycaemic disorders are provided and so also in Patent number WO9843484.

In US Patent number 6043824, a herbal composition for the treatment of Diabetes comprising therein of *Trigonella foenum-graecum, Nigella sativa, Origanum vulgare, Rosmarinus officinalis, Lupinus termis, Lawsonia inermis* and *Foeniculum vulgare* in a capsule form has been claimed. US Patent number 5886029 alludes to a medicinal invention, which results in a significant reduction in serum glucose due to the regeneration of pancreatic islet cells. The medicinal composition includes Gymnemic acid, *Cinnamomum tamala, Syzgium cumini, Trigonella foenum graceum, Azardichta indica, Ficus racemosa* and *Tinospora cordifolia.* This combination leads to a regeneration of pancreas cells, which then start producing insulin on their own.

Patent number WO0172316 describes an edible ayurvedic herbal composition for reducing the blood sugar level in humans suffering from Diabetes *mellitus* comprising therein, a mixture of ingredients selected from *Cinnamomum zeylanicum, Artocarpus heterophyllus, Salacia reticulata, Tinospora cordifolia* and *Pterocarpus marsupium* in therapeutically effect proportions to treat abnormal levels of blood sugar and Diabetes *mellitus.*

US Patent number 5917052 states that no prior study has described any hypoglycaemic activity or extracts of *Cryptolepis* sp or that quindoline alkaloids such as cryptolepine or quindoline would be useful as hypoglycaemic agents. The invention provides a method for the use of extracts from *Cryptolepis* sp and compounds of the quindoline family of alkaloids such as quindoline, cryptolepine etc. as well as pharmaceutically acceptable salts thereof as hypoglycaemic agents or as agents to lower triglyceride levels, particularly in diabetic subjects.

In US Patent number 5837255, it has been held that no prior study has described any hypoglyemic activity of extracts of *Harungana* spp or *Vismia* spp nor was there any prior suggestion that anthracenone compounds such as harunganin or vismin are useful as hypoglycaemic agents. The invention provides a method for the use of extracts from *Harunganin* spp or from *Vismia* spp and for the use of anthracenone compounds harunganin and vismin, as well as pharmaceutically acceptable salts thereof as hypoglycaemic agents or agents to lower blood glucose levels, particularly in diabetic subjects.

US Patent number 2002041904 has mentioned that in recent years among therapeutic drugs classified as anti diabetic agents, alpha glucosidase inhibitors which inhibit the activity of alpha-glucosidase have been widely used in the treatment of Diabetes and pre-Diabetes. *Salacia reticulata* has been used since ancient times in the ancient medicines of India and Sri Lanka. The object of this invention has been to provide a novel compound which is extracted from the woody climbing plants, *Salacia prinoides* and *Salacia oblonga* and is superior in terms of its characteristic of inhibiting the activity of alpha-glucosidase (compound being referred to as 'alpha-glucosidase inhibitor'). In US Patent number 5691386, it has been reported that plant of the genus *Salacia* has been used to treat Diabetes. In India, a hot water extraction of the whole plant *Salacia prinoides* has been taken orally as an anti-diabetic (P.N. Mehra et al., Res. Bull Punjab University Sci 20: 487-502 (1969). In Sri Lanka aqueous extracts of the roots of *Salacia reticulata* have been used in the treatment of Diabetes *mellitus* (E.H. Karunanayake et al., J. Ethnopharmacol 13 (2): 227-228 (1985). This invention claims to provide a novel triterpenoid compound, 3-beta,30–dihydroxylup-20-29-en-2 one as well as pharmaceutically acceptable salts thereof, having hypoglycaemic activity, hypoglycemic compositions comprising the novel triterpenoid

compound in purified form as well as methods for their use, as an hypoglycaemic agent. The invention further encompasses compositions comprising the triterpenoid compounds in purified form or pharmaceutically acceptable salts for use as hypoglycaemic agent, useful for the treatment of Diabetes.

US Patent number 5916567 relates to a herbal therapeutic product for treating Diabetes and relates to a therapeutic product processed from the seed of a plant from the family Leguminosea, whose fibres affect the blood sugar level by increasing the viscosity of the unstirred layer between food and the lining of the intestines and the stomach thereby making the carbohydrates available for absorption at a slower rate.

US Patent number 5,470,873 discloses a composition for the treatment of NIDDM comprising therein, maltol, obtained from Ginseng roots and an extract obtained from Orthosiphon aristatus, effective in regulating blood glucose levels in diabetic animals but this by itself is not sufficient to normalise the glucose levels, nor was any lasting effect determined following termination of the treatment.

The present invention relates to an amalgam of 15 (Fifteen) different traditional Indian medicinal plants, the compounds of which have been identified and isolated in order to have the same screened to ascertain their therapeutic effects in treating Diabetes, more specifically type II Diabetes with the compositions derived from non-toxic medicinal plants and effective in the prevention, treatment and cure of NIDDM.

Brief description of the tables and figures:

Table 1 gives the list of medicinal plants used to arrive at the formulation, AGT_D_For_0001.

Table 2 gives the list of extracts from individual plants.

Table 3 shows a representative mass spectral peak grid arrived at using a comparative mass spectral peak analysis approach that illustrates the components that are either common or unique to individual extracts.

Table 4 shows the comparative results of the mass spectrometry analysis of the chloroform-methanol fractions of formulation AGT_D_For_0001_05:03 and its constituents, AGT_D_Mch_Se_05:03, AGT_D_Tfg_Se_05:03, AGT_D_Ej_Se_05:03 & AGT_D_Gs_Ml_05:03 indicating the common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z)

Table 5 shows the comparative results of the mass spectrometry analysis of the methanolic fractions of formulation AGT_D_For_0001_05 and its constituent plants, AGT_D_Mch_Se_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05 and AGT_D_Gs_Ml_05.

Table 6 fives an estimate of total number of constituents contained in a few of the individual plant extracts that have been characterized using HPLC-based metabolite fingerprinting.

Table 7 summarizes the comparative HPLC-based fingerprinting analysis done in the case of different plant extracts using a few representative instances (Ma_Le_Wa_01 v/s Ws_Ro_Wa_01; Ca_Ro_Et_01 v/s Ws_Ro_Et_01 and Ej_Se_Me_01 v/s Tt_Fr_Me_01).

Figure 1 (A-F) gives the representative mass spectra of methanolic extracts (AGT_D_Gs_Ml_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05) and chloroform extracts (AGT_D_Gs_ML_03, AGT_D_Tfg_Se_03, and AGT_D_Ej_Se_03)

Figure 2 (A-E) gives the representative mass spectra of the comparative mass spectrometric analysis between the chloroform fractions of the formulation, AGT_D_For_0001_03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_03, AGT_D_Ej_Se_03, AGT_D_Tfg_Se_03 and AGT_D_Gs_Ml_03.

Figure 3 (A-E) gives the representative mass spectra of the comparative mass spectrometric analysis between the chloroform –methanol fractions of the formulation, AGT_D_For_0001_05:03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_)5:03, AGT_D_Ej_Se_)5:03, AGT_D_Tfg_Se_05:03 and AGT_D_Gs_Ml_05:03.

Figure 4 (A-E) gives the representative mass spectra of the comparative mass spectrometric analysis between the methanol fractions of the formulation, AGT_D_For_0001_05 and the methanol extracts of a few of its constituents, AGT_D_Mch_Se_05, AGT_D_Ej_Se_05, AGT_D_Tfg_Se_05 and AGT_D_Gs_Ml_05.

Figure 5 gives the chromatograms of extracts Ma_Le_Wa_01 and Ws_Ro_Wa_01 at selected wavelengths

Figure 6 gives the chromatograms of extracts Cr_Ro_Et_01 and Ws_Ro_Et_01 at selected wavelengths

Figure 7 gives the chromatograms of extracts Ej_Se_Me_01 and Tt_Fr_Me_01 at selected wavelengths

Description

The object of the invention is to provide edible herbal dietary supplements. The invention relates to a method and a composition for potentiating insulin activity to treat Diabetes patients. This composition has an effect on smoothing out fluctuations in the glucose levels.

The insulin potentiating agents are natural substances derived from plant extracts and can be safely consumed by humans. This naturally derived agent has an advantage in that it does not cause side effects. These agents can be used with conventional drug treatments like oral hypoglycemic agent or insulin.

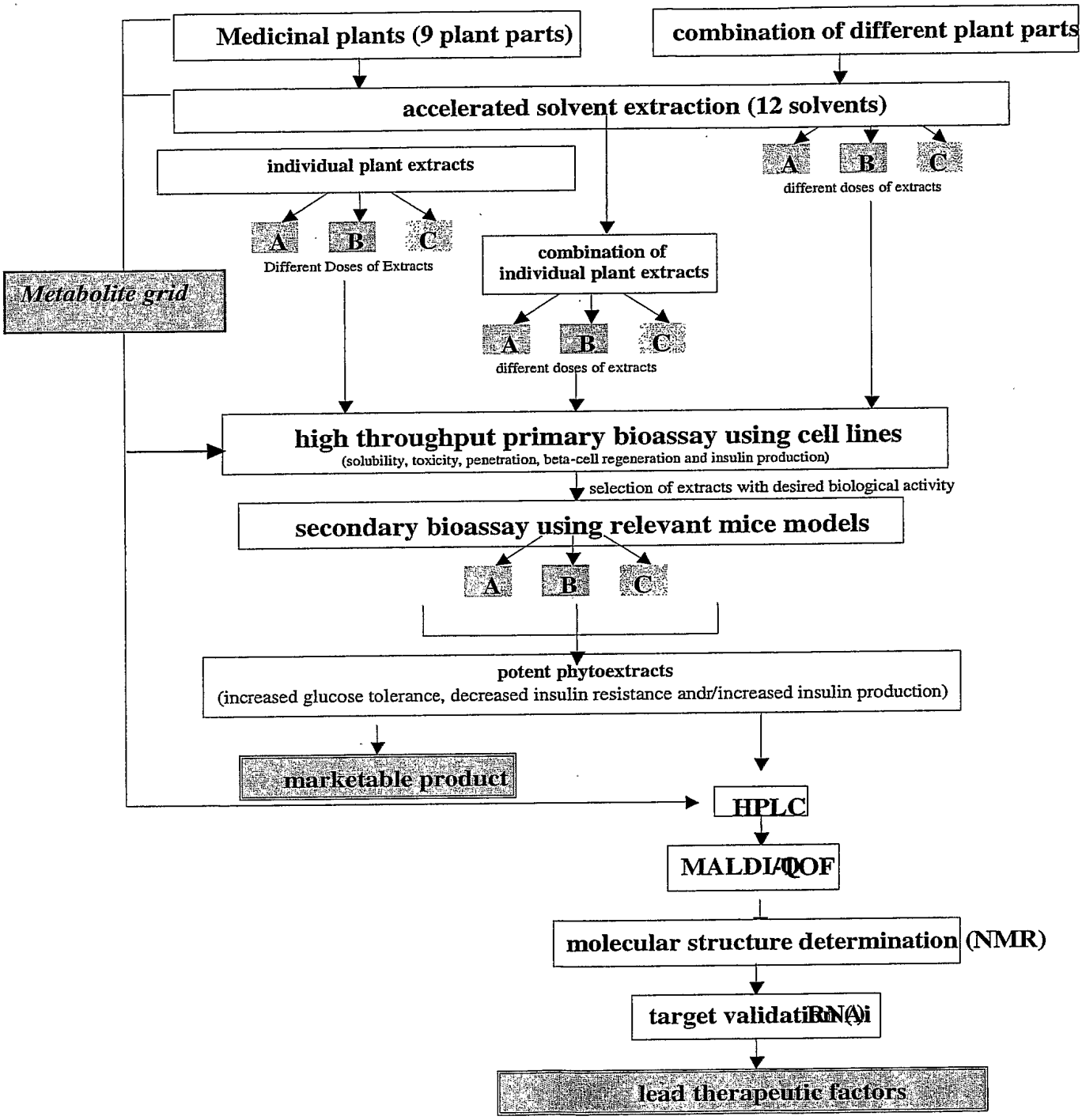
One of the main problems that have been reported in the case of phyto-formulations is the lack of clarity in terms of comprehensive qualitative and quantitative characterization of all the detectable components present in the mixture. The availability of such information about phyto-extracts will play a major role in the scientific validation and standardization of both the therapeutic effects and constituents present in these phytoextracts.

Metabolite profiling has emerged as a robust tool that is fast, reliable, sensitive and suitable for automation, covering a significant number of metabolites. A range of analytical technologies enhances the sensitivity and universality of mass spectrometry by chromatographic separations. Although the use of multi- target profiling had been earlier limited to rapid clinical detection of human diseases, metabolic screening approaches using mass spectrometry are being increasingly used in plant research at present. A major advantage of mass-spectrometry is that unknown peaks can be determined as reliably as known target analytes without prior knowledge of their exact chemical structure. Studies using gas chromatography/mass spectrometry (GC/MS) have automatically quantified 326 distinct compounds from *Arabidopsis thaliana* leaf extracts. It has been possible to assign a chemical structure to approximately half of these compounds. Comparison of four *Arabidopsis* genotypes (two homozygous ecotypes and a mutant of each ecotype) showed that each genotype possesses a distinct metabolic profile. Data mining tools such as principal component analysis enabled the assignment of "metabolic phenotypes" using these large data sets. The results of this study have shown that metabolic phenotypes of the two ecotypes were more divergent than were the metabolic phenotypes of the single-loci mutant and their parental ecotypes. These results demonstrate the use of metabolite profiling as a tool to significantly extend and enhance the power of existing functional genomics approaches. Due to the increased chemical complexity and diversity at the metabolite level in higher plants, no singular technique exists for profiling all cellular metabolites concurrently. This problem can be approached through the division of metabolites into major profiling classes, *i.e.* triterpenoids, phenolics, lipids, carbohydrates, amino acids and carbohydrates. The hyphenated mass spectrometric techniques such as GC/LC/ESI- MS provide both relative quantitative abundances and specific information that can be utilized in chemical identification. Methods have been developed using HPLC interfaced with an ion trap mass spectrometer capable of sequential tandem mass spectrometry for profiling plant metabolites, *i.e.* HPLC-ESI-MS^n. This approach has been used to profile saponin glycosides in multiple cultivars of alfalfa followed by the comparison of these profiles to the model legume *M. truncatula*. To date, twenty-seven novel saponin glycosides in *M. truncatula* have been identified using this technology. This technology was also used to identify novel malonated saponin glycosides in alfalfa and *M. truncatula*.

Metabolite grid (*Metagrid*)

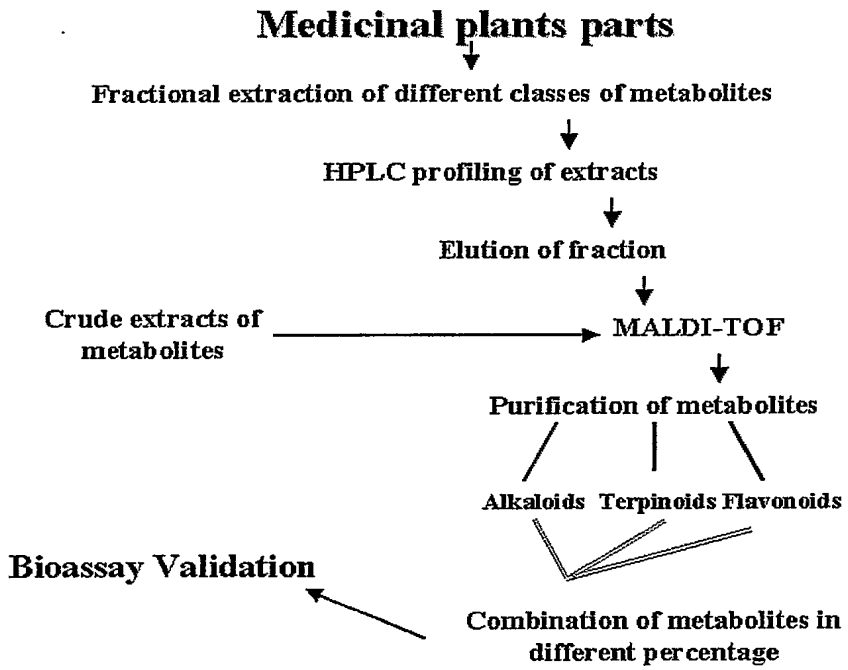
The plants selected for the isolation of therapeutically relevant extracts/molecules ("Yezdex") to be used in the treatment of Diabetes, are being subjected to both targeted and non-targeted screening procedures. The ongoing-targeted screening procedures, which feature a comprehensive metabolite profiling of multitudes of phyto-extracts, were envisaged to facilitate the creation of a metabolite grid. Extensive comparative analyses of the individual plant species with the existing drug and/or phytoextract formulations in the market has revealed the presence of both unique and common molecular constituents that can be used individually and/or in combination to accelerate the process of discovery of novel therapeutic formulation.

Flow Chart 1: Avestha's *Metagrid* to achieve relevant therapeutic extracts/molecules.



Screening Methodologies:

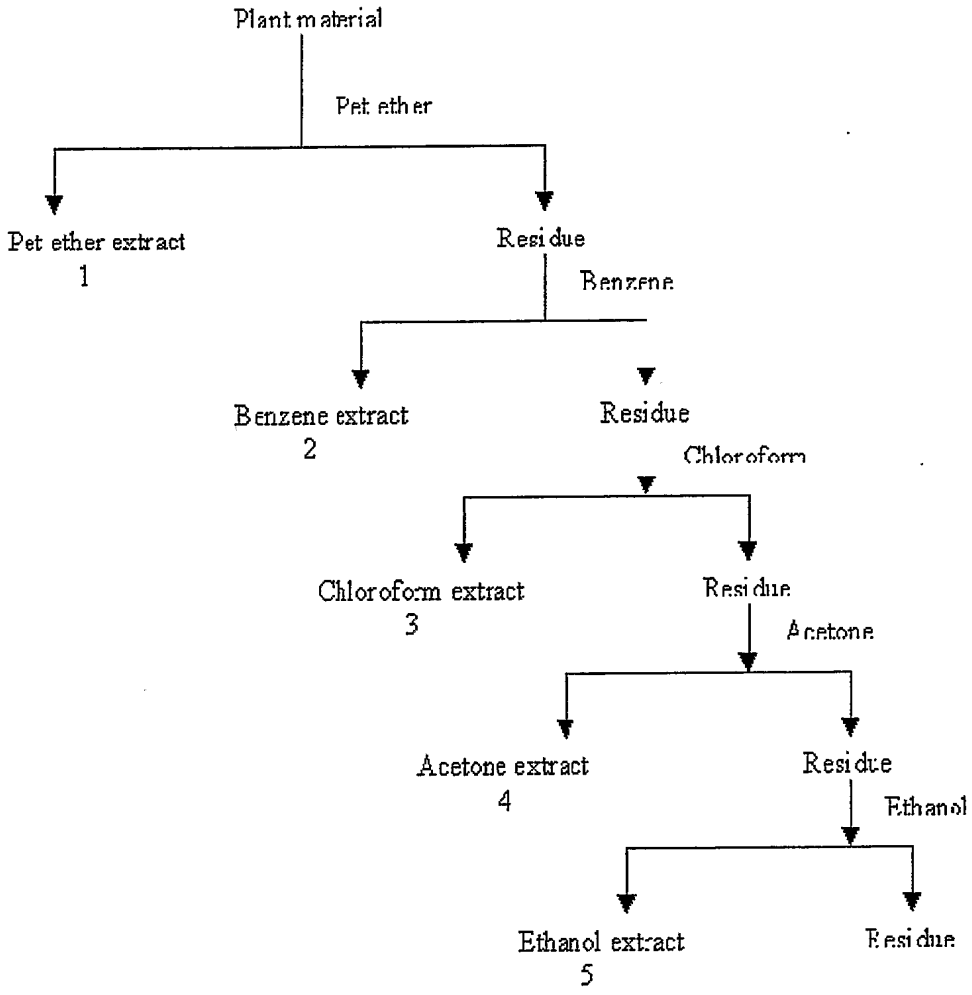
Flow Chart 2: Extraction process used to arrive at the *Metagrid*



Extraction:

Method 1: The successive extraction from various medicinal plants parts was carried out using soxhlet extractor. The solvents used, were based on their sequential polarity starting from non-polar to polar, wherein, various classes of metabolites were extracted viz; petroleum ether (phytosterols, fixed oils and fats), benzene (fixed oils and fats), chloroform (alkaloids), acetone (phytosterols, phenolics and tannins) ethanol (alkaloids, carbohydrates, glycosides, phytosterols, saponins, phenolics, tannins, proteins and amino acids) and water (alkaloids, carbohydrates, glycosides, saponinns, phenolics, tannins, proteins, amino acids, gums and mucilage) at 65°C. These fractions were lyophilized and stored in amber colored bottles at 4°C.

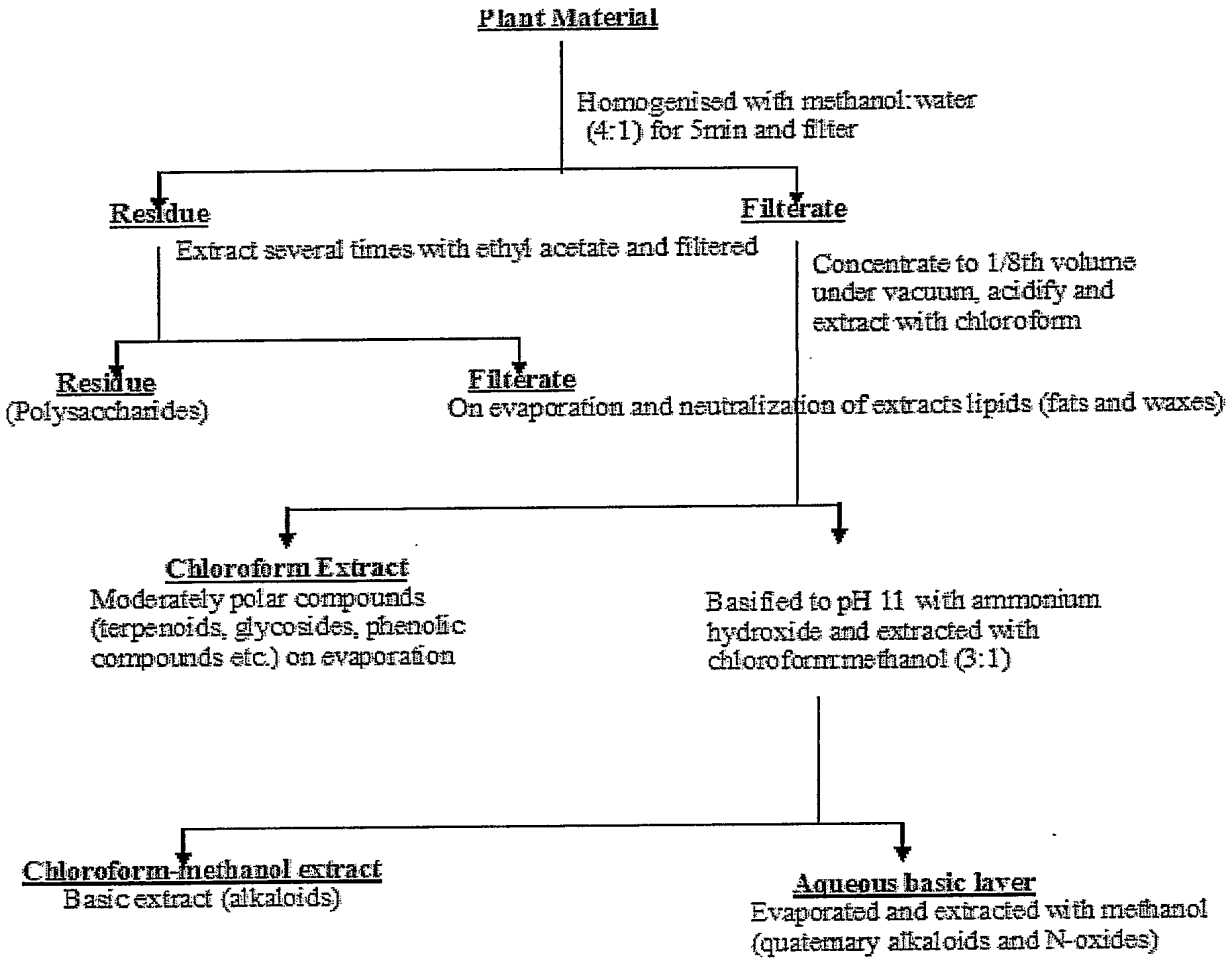
Flow Chart 3: Successive Extraction Process



Phytochemical investigations were also carried out on these extracts using various tests like Mayer's and Dagendorf's tests for alkaloids; Molisch, Fehling and Benedict tests for carbohydrates; Lieberman Buchard's test for phytosterols and triterpenes; spot test for fixed oils and fats; Ferric chloride and Lead acetate test for phenolic compounds and tannins; Ninhydrin and Biuret tests for protein and aminoacids; alcoholic precipitation followed by Molisch test for gum and mucilages.

Method 2: To characterize a particular class of metabolites, fractional extraction procedure was adopted. In this method, various metabolite classes were screened like polysaccharides, terpenoids, phenolics, alkaloids, oils, fat and waxes present in the medicinal plants parts. The flow chart of the procedure is depicted below:

Flow Chart 4: Fractional Extraction Procedure



High Performance Liquid Chromatography ("HPLC") profiling:

The extracted fractions were subjected to HPLC using μ bondapak C $_{18}$ column (Waters Alliance 2695 Separation Module) to separate the constituent metabolites. The fractions were eluted using a combination (80:20, 60:40, 50:50, 40:60, 20:80) of methanol:water / acetonitrile:water. The gradient run was also carried out wherever required. 5- 10ul of sample was injected with flow rate of 1ml/min

and HPLC run was performed for 30 minutes. The detection was carried out on photodiode array and the analysis of the results was done with the help of Millennium™ software.

Identification and characterization of purified/partially purified extracts by MALDI TOF:

The metabolites were identified and characterized by using the MALDI –TOF Voyager system 4266. The matrix for MALDI-TOF used was alpha cyano-4-hydroxycinnamic acid. Nuclear Magnetic Resonance (NMR) will be performed for unique and common fallouts of *Metagrid* for it structure elucidation.

Comprehensive constituent profiling and creation of the Metagrid:

A comparative profile of a therapeutic formulation and its individual constituents has been worked upon. AGT_D_For_0001 (See Table 1) , a therapeutic formulation, which comprises of approximately 15 (Fifteen) medicinal plants and their comparative analyses has been undertaken using mass spectrometry (MALDI-TOF MS). In addition to the formulation, 51 (Fifty One) individual plant extracts (see Table 2) have been comprehensively profiled using HPLC. Representative analytical data of the biochemical profiling carried out thus far is shown below:

Mass-spectrometry based comprehensive constituent profiling of the formulation AGT_D_For_0001 and the constituent plants

Example1: The methanolic and ethanolic extracts of Tfg, Ej and Gs were analysed using the screening method 1 as described earlier. The methanolic extracts AGT_D_Gs_Ml_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05, and chloroform extracts AGT_D_Gs_ML_03, AGT_D_Tfg_Se_03, AGT_D_Ej_Se_03 when compared, revealed common mass spectral peaks in both the solvents, viz. m/z 104, 112, 176, 184, 212, 228, 241, 496, 522, 592, 606. (As shown in Table 3 and Figure 1) It was also observed that molecular mass spectral peaks m/z 203, 267, 337, 634, 694 were unique to the methanolic extracts and molecular mass spectral peaks m/z 190, 336, 340, 390, 520, 621 were unique to the chloroform extracts.

Example2: A comparative profiling of the therapeutic formulation, AGT_D_For_0001, and its individual constituents, mentioned supra, was carried out to isolate terpenoids/ phenolics, using method 2 as previously described. The comparative results of the mass spectrometry analysis between the chloroform fractions of the formulation, AGT_D_For_0001_03 and its constituents, AGT_D_Mch_Se_03, AGT_D_Ej_Se_03, AGT_D_Tfg_Se_03, AGT_D_Gs_Ml_03 revealed a few common mass spectral peaks m/z 104, 138, 172, 184, 336, indicating the presence of terpenoids/ phenolics which may play a significant role in the treatment of diseases. The mass spectral peaks such as m/z- 212, 288, 338, 496.3, 520.39, 623.48 that are present in formulation but are also uniquely present in few of the medicinal plant parts analysed thus far indicates, that this system can be utilized to track the origin of these specific compounds to specific plants or plant parts used to arrive at a therapeutic formulation. (As shown in Figure 2)

Example 3: The basic alkaloids were extracted by method 2 and were profiled by HPLC and mass spectrometry. The comparative results of the mass spectrometry analysis the chloroform-methanol fractions of formulation AGT_D_For_0001_05:03 and its constituents, AGT_D_Mch_Se_05:03, AGT_D_Tfg_Se_05:03, AGT_D_Ej_Se_05:03, AGT_D_Gs_Ml_05:03 reveals the common mass, m/z 104, 138, 172, 184, 336 and is suggestive of presence basic alkaloids and its precursors, which may have significant role in the treatment of diseases. As mentioned in example 2, the mass spectral peaks such as m/z- m/z 112, 155, 212, 286, 288, 338, 352.16, 496.38, 520.39, 623.48 that are present in formulation but are also uniquely present in few of the medicinal plant parts analyzed thus far indicates, that this system can be utilized to track the origin of these specific compounds to specific plants or plant parts used to arrive at a therapeutic formulation. (As shown in Table 4 & Figure 3)

Example 4: The quaternary alkaloids were extracted by method 2 and were profiled by HPLC and mass spectrometry. The comparative results of the mass spectrometry analysis of the methanolic fractions of formulation AGT_D_For_0001_05 and its constituent plants, AGT_D_Mch_Se_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05 and AGT_D_Gs_Ml_05 revealed the presence of common mass spectral peaks m/z 104, 172, 190, 212, 228, 294, 379 that are representative of quaternary alkaloids, N-oxides and their precursors. As mentioned in example 2 and 3, the mass spectral peaks such as m/z- 118.1, 138.06, 241.17, 250.1, 265.97, 296, 345, 441.05, 443.04, 492.98 that are present in formulation but are also uniquely present in few of the medicinal plant parts analyzed thus far indicates, that this system can be utilized to track the origin of these specific compounds to specific plants or plant parts used to arrive at a therapeutic formulation. (As shown in Table 5 & Figure 4)

HPLC based comprehensive constituent profiling and metabolite fingerprinting of individual plant extracts:

The extracted fractions were subjected to HPLC using μ bondapak C_{18} column (Waters Alliance 2695 Separation Module) to separate the constituent metabolites. The fractions were eluted using a combination (80:20, 60:40, 50:50, 40:60, 20:80) of methanol:water / acetonitrile:water. The gradient run was also carried out wherever required. 5- 10ul of sample was injected with flow rate of 1ml/min and HPLC run was performed for 30 minutes. The detection was carried out on photodiode array and the analysis of the results was done with the help of Millennium™ software.

Comprehensive constituent profiling and metabolite fingerprinting of individual 51 individual plant extracts (see table 2) has been carried out. (See Table 6 and Figure 5)

Comparative metabolite fingerprinting analysis has revealed the presence of both common and unique constituents that are present in individual plant extracts that have been extracted under similar conditions. (See Table 7)

The intuitive methodology used to arrive at the data (using a combinatorial matching of retention times and absorption maxima) summarized in table 7 is illustrated using a few representative instances. shown in Figure 6 & 7.

The extracts were randomly screened for metabolites by HPLC and mass spectrometry, the results reveal the group of common metabolites in most of the extracts and some unique molecular masses were also observed which would be further subjected to structural elucidation and characterization by MS^n and NMR. Furthermore, derivatisation of these characterized molecules will be carried out using the synthetic chemistry approach.

Carbon dioxide extraction procedure would be carried as against the conventional method of extraction, expecting more efficient extraction with less solvent consumption and shorter extraction time.

Screening of Extracts for its bioactivity:

51 (Fifty One) individual extracts and their combinations are ready for primary and secondary assay systems for Diabetes.

The extracts will be tested for primary assay:

a. using murine pancreatic islet cell lines (***HIT, HIP, RIN, alpha-TC and beta-TC***) to monitor the changes in levels of insulin-secretion in response to the treatment with defined phyto-extracts
b. changes in insulin-resistance will also be monitored in the murine adipocyte cell line- ***3T3-L1*** in response to the treatment with defined phytoextracts.

Secondary bioassay using mouse models will be conducted to validate the phytoextracts. Different kinds of mouse models will be used for this purpose

a. streptozotocin (STZ) /alloxan induce diabetic mouse.
b. Genetically modified mouse models (db/db,db/ob,and ob/ob)

The gene for ORCA-3, a jasmonate responsive APETALA2 (AP2) domain transcription factor may be isolated by transferred DNA activation tagging and expressed in these therapeutic plants expecting an enhanced expression of several metabolites biosynthetic genes and consequently, an increased accumulation of secondary metabolites of interest in the treatment of diabetes and allied disorders.

Table 1: List of medicinal plants used to arrive at the formulation, AGT_D_For_0001

Formulation AGT_D_For_0001		Botanical name
1	Amlaki	*Phyllanthus emblica*
2	Guduchi	*Tinospora cordifolia*
3	Nimbha	*Azadiractha indica*
4	Jambu	*Eugenia jambulana*
5	Medhika	*Trigonella foenum graceum*
7	Haritaki	*Terminalia chebula*
8	Vibhitaki	*Mucunapuriens*
9	Haridra	*Curcuma longa*
10	Udumbara	*Ficus glomerata*
11	Bhumyamalaki	*Phyllanthus niruri*
12	Ashwagandha	*Withania somenifera*
13	Karavalli	*Momordica charantia*
14	Meshasringi	*Gymnema sylvestre*
15	Silajit	*Euphorbia royleana*

Table 2: List of extracts from individual plants

Extract ID	*Plant name*	*Tissue*	*Solvent*
Cr_Ro_Me_01	*Catharanthus roseus*	Root	methanol
Cr_Ro_Et_01	*Catharanthus roseus*	Root	ethanol
Cr_Ro_Ch_01	*Catharanthus roseus*	Root	chloroform
Ej_Se_Me_01	*Eugenia jambolana*	Seed	methanol
Ej_Se_Et_01	*Eugenia jambolana*	Seed	ethanol
Ej_Se_Ch_01	*Eugenia jambolana*	Seed	chloroform
Ej_Se_Pe_01	*Eugenia jambolana*	Seed	petroleum ether
Ej_Se_Be_01	*Eugenia jambolana*	Seed	benzene
Ej_Se_Et_01(20)	*Eugenia jambolana*	Seed	ethanol-20%
Ej_Se_Wa_01	*Eugenia jambolana*	Seed	water
Eo_Fr_Me_01	*Emblica officinalis*	Fruit	methanol
Eo_Fr_Ch_01	*Emblica officinalis*	Fruit	chloroform
Gs_Le_Me_01	*Gymnema sylvestre*	Leaf	methanol
Gs_Le_Et_01	*Gymnema sylvestre*	Leaf	ethanol
Gs_Le_Ch_01	*Gymnema sylvestre*	Leaf	chloroform
Gs_Le_Pe_01	*Gymnema sylvestre*	Leaf	petroleum ether
Gs_Le_Be_01	*Gymnema sylvestre*	Leaf	benzene
Gs_Le_Et_01(20)	*Gymnema sylvestre*	Leaf	ethanol-20%
Gs_Le_Wa_01	*Gymnema sylvestre*	Leaf	water
Ma_Le_Me_01	*Melia azadirechta*	Leaf	methanol
Ma_Le_Et_01	*Melia azadirechta*	Leaf	ethanol
Ma_Le_Ch_01	*Melia azadirechta*	Leaf	chloroform
Ma_Le_Pe_01	*Melia azadirechta*	Leaf	petroleum ether
Ma_Le_Be_01	*Melia azadirechta*	Leaf	benzene
Ma_Le_Hx_01	*Melia azadirechta*	Leaf	hexane
Mc_Fr_Me_01	*Morinda citrifolia*	Fruit	methanol
Mc_Fr_Et_01	*Morinda citrifolia*	Fruit	ethanol
Mc_Fr_Ch_01	*Morinda citrifolia*	Fruit	chloroform
Pm_Ba_Et-01	*Pterocarpus marsupium*	Bark	ethanol
Pm_Ba_Wa-01	*Pterocarpus marsupium*	Bark	water
Tc_Fr_Me_01	*Tinospora cardifolia*	Fruit	methanol
Tc_Fr_Et_01	*Tinospora cardifolia*	Fruit	ethanol
Tc_Fr_Ch_01	*Tinospora cardifolia*	Fruit	chloroform
Tt_Fr_Me_01	*Tribulus teristris*	Fruit	methanol
Tt_Fr_Et_01	*Tribulus teristris*	Fruit	ethanol
Tt_Fr_Ch_01	*Tribulus teristris*	Fruit	chloroform
Tt_Fr_Pe_01	*Tribulus teristris*	Fruit	petroleum ether

Tt_Fr_Be_01	*Tribulus teristris*	Fruit	benzene
Tfg_Se_Me_01	*Trigonella foenum graecum*	Seed	methanol
Tfg_Se_Et_01	*Trigonella foenum graecum*	Seed	ethanol
Tfg_Se_Ch_01	*Trigonella foenum graecum*	Seed	chloroform
Tfg_Se_Pe_01	*Trigonella foenum graecum*	Seed	petroleum ether
Tfg_Se_Be_01	*Trigonella foenum graecum*	Seed	benzene
Tfg_Se_Et_01(20)	*Trigonella foenum graecum*	Seed	ethanol-20%
Tfg_Se_Wa_01	*Trigonella foenum graecum*	Seed	water
Ws_Ro_Me_01	*Withania somnifera*	Root	methanol
Ws_Ro_Et_01	*Withania somnifera*	Root	ethanol
Ws_Ro_Ch_01	*Withania somnifera*	Root	chloroform
Ws_Ro_Pe_01	*Withania somnifera*	Root	petroleum ether
Ws_Ro_Be_01	*Withania somnifera*	Root	benzene
Ws_Ro_Wa_01	*Withania somnifera*	Root	Water

Table 3 shows a representative mass spectral peak grid arrived at using a comparative mass spectral peak analysis approach that illustrates the components that are either common or unique to individual extracts.

Molecular Masses of constituents (m/z)common to both methanolic and ethanolic extracts	Molecular Masses of components (m/z), unique to Methanolic extracts	Molecular Masses of components (m/z), unique to chloroform extracts
104	203	190
112	267	336
176	337	349
184	534	390
212	594	520
228		621
241		
496		
522		
558		
592		
606		

Table 4 Comparative results of the mass spectrometry analysis of the chloroform-methanol fractions of formulation AGT_D_For_0001_05:03 and its constituents, AGT_D_Mch_Se_05:03, AGT_D_Tfg_Se_05:03, AGT_D_Ej_Se_05:03 & AGT_D_Gs_Ml_05:03 indicating the common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z)

Common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z)	Mass spectral peaks that are uniquely present in the constituent plants (m/z)	
104	112	Ej
138	155	Gs
172	212	Gs, Ej
184	286	Ej
336	288	Tfg
	338	Ej
	352.16	Mch, Ej
	496.38	Mch, Tfg
	520.39	Mch, Tfg
	623.48	Mch, Ej, Gs

Table 5 The comparative results of the mass spectrometry analysis of the methanolic fractions of formulation AGT_D_For_0001_05 and its constituent plants, AGT_D_Mch_Se_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05 and AGT_D_Gs_Ml_05.

Common mass spectral peaks occurring both in the formulation and the constituent plants masses (m/z))	Mass spectral peaks that are uniquely present in the constituent plants (m/z)	
104.1	118.1	Tfg
	138.06	Mch, Tfg
172.05	241.17	Mch, Tfg
190.07	250.1	Ej
207.1	265.97	Ej, Mch, Gs
212.05	296	Gs
228.01	342	Ej
294.1	441.05	Ej, Tfg, Gs
379.1	443.04	Ej, Gs
	492.98	Gs

Table 6: Estimation of total number of constituents contained in a few of the individual plant extracts that have been characterized using HPLC-based metabolite fingerprinting.

Extract ID		Constituents in		Total number of constituents
		UV Range	Visible Range	
1	Eo_Fr_Me_01	123	603	726
2	Cr_Ro_Me_01	232	674	906
3	Ws_Ro_Me_01	247	590	837
4	Tfg_Se_Me_01	176	604	780
5	Ej_Se_Me_01	150	656	806
6	Tt_Fr_Me_01	193	507	700
7	Mc_Fr_Me_01	169	615	784
8	Tc_Fr_Me_01	226	561	787
9	Ws_Ro_Et_01	218	598	816
10	Tfg_Se_Et_01	152	616	768
11	Ws_Ro_Wa_01	159	618	777
12	Ma_Le_Et_01	221	540	761
13	Cr_Ro_Et_01	229	533	762
14	Ej_Se_Et_01	109	600	709
15	Gs_Le_Et_01	191	552	743
16	Gs_Le_Me_01	237	557	794
17	Ma_Le_Wa_01	114	643	757

Table 7 summarizes the comparative HPLC-based fingerprinting analysis done in the case of different plant extracts using a few representative instances (Ma_Le_Wa_01 v/s Ws_Ro_Wa_01; Ca_Ro_Et_01 v/s Ws_Ro_Et_01 and Ej_Se_Me_01 v/s Tt_Fr_Me_01).

Extract ID		Total No. of constituents	Common Constituents	Unique constituents
1	Ma_Le_Wa_01 Ws_Ro_Wa_01	757 777	7	750 770
2	Ca_Ro_Et_01 Ws_Ro_Et_01	762 816	41	721 775
3	Ej_Se_Me_01 Tt_Fr_Me_01	806 700	40	766 660

CLAIMS

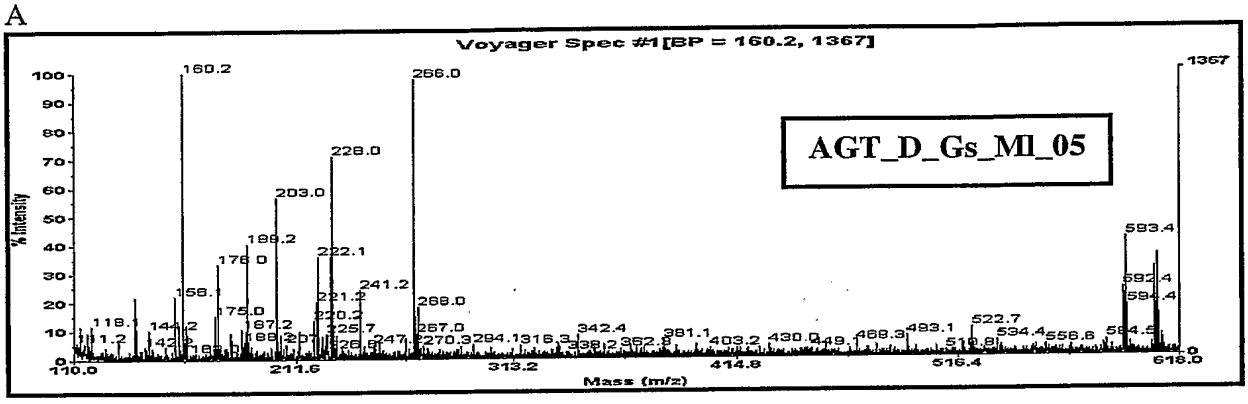
1. A screening method of arriving at a formulation, comprising of a combination of various herbal extracts from potent Indian medicinal plants, effective in the treatment and cure of Diabetes and its associated problems like cardiac diseases, renal failure, eye diseases, neuropathy, obesity and a host of other diseases.
2. A claim as in claim 1 wherein the metabolites present in the formulation can be used individually as a drug molecule.
3. A claim as in claim 2 wherein the whole combination of metabolites present in the formulation can be used a drug molecule.
4. A claim as in claim 3 wherein the formulation of a drug molecule can be arrived at by different metabolite class combinations.
5. A claim as in claim 1 wherein each individual herbal extract exhibiting properties can play a significant role in the treatment and cure of diabetes and its associated problems.
6. A claim as in claim 5 wherein the extracts can be used in various combinations to play an effective role to provide a synergistic effect in the treatment of diabetes and related risks.
7. A claim as in claim 5 wherein the metabolites present in each individual extract can be used as a therapeutic agent to treat diabetes and allied disorders.
8. A claim as in claim 5 wherein the combination of various metabolites from the different extracts can be used as a drug molecule in treating diabetes and associated risks.
9. A claim as in claim 1 wherein the screening method adopted is effective in the isolation, identification and characterisation of molecules in isolation.
10. A claim as in claim 1 wherein the screening method adopted can play a useful role in the isolation, identification and characterization of a therapeutic class of molecules.
11. A claim as in claim 9 wherein the molecule identified, can be applied for derivitisation of analogues.
12. A claim as in claim 11 wherein the derivitised molecule renders greater efficacy in treating Diabetes and allied disorders.
13. A claim as in claim 1 wherein the formulation can be administered sequentially and/or simultaneously.
14. A claim as in claim 13 wherein the formulation is rendered suitable for oral and peritoneal including subcutaneous, intramuscular, intravenous and intradermal administration.
15. A claim as in claim 14 wherein the dosage can be administered in the form of tablets, pills, powders, solutions, syrups, suspensions, emulsions, granules, capsules and suppositories.

ABSTRACT

The present invention relates to a unique method, *Metagrid,* for selecting plant metabolites and their constitutions into potent extracts "Yezdex" associated with a specific disease, in this case, Diabetes. Using 15 (Fifteen) medicinal plants of Indian origin, 51 (Fifty One) unique extracts containing therapeutically relevant molecules were isolated and scientifically characterized, using advanced technologies to be used in the treatment of Diabetes. This invention also relates to an edible composition comprising of 15 (Fifteen) Indian herbal extracts which can be used as a dietary supplement/drug and also useful in lowering the glucose levels in the blood of mammals, particularly humans, suffering from Diabetes *mellitus*.

Figure 1 (A-F): Representative mass spectra of methanolic extracts (AGT_D_Gs_Ml_05, AGT_D_Tfg_Se_05, AGT_D_Ej_Se_05) and chloroform extracts (AGT_D_Gs_ML_03, AGT_D_Tfg_Se_03, and AGT_D_Ej_Se_03)

A



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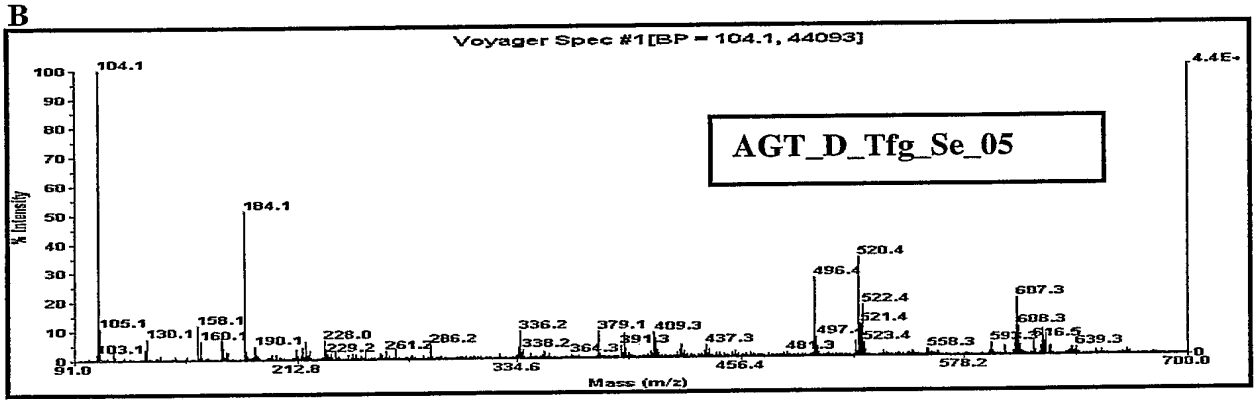

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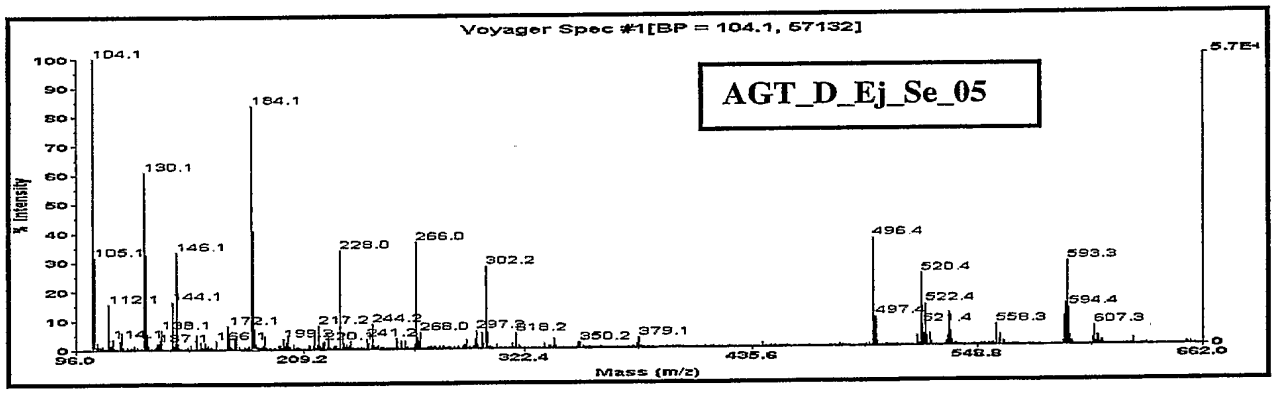
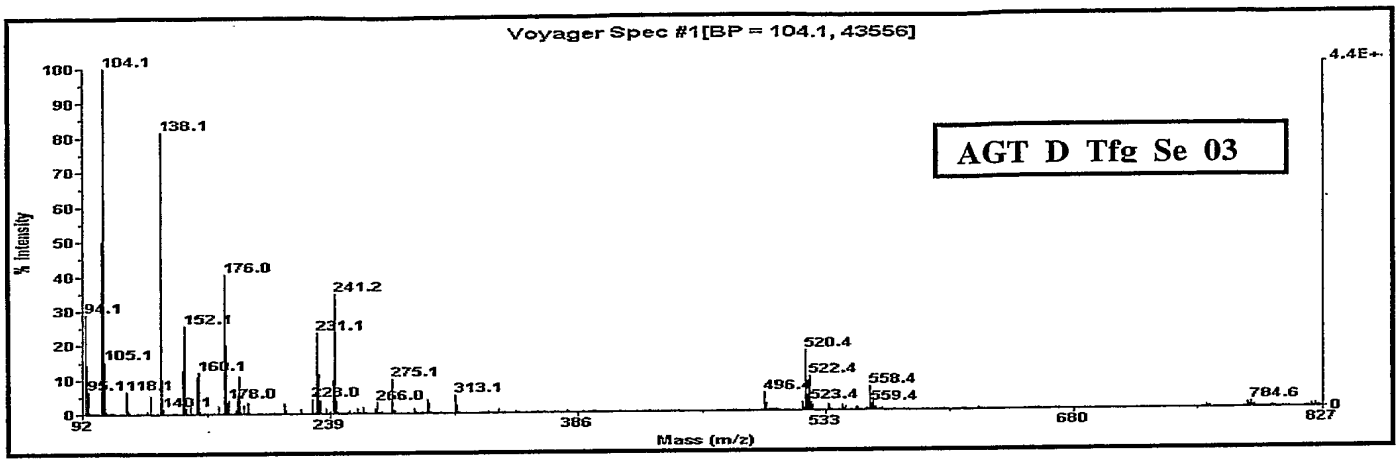
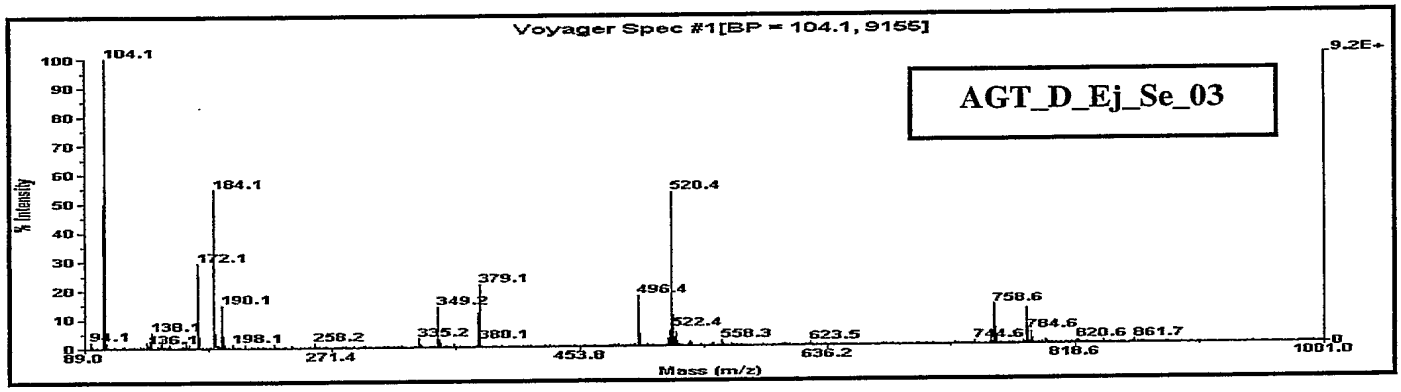



Voyager Spec #1[BP = 104.1, 43556]
AGT D Tfg Se 03
% Intensity
Mass (m/z)
104.1
138.1
176.0
241.2
94.1
152.1
231.1
105.1
160.1
275.1
520.4
522.4
313.1
496.4
558.4
784.6
4.4E+

D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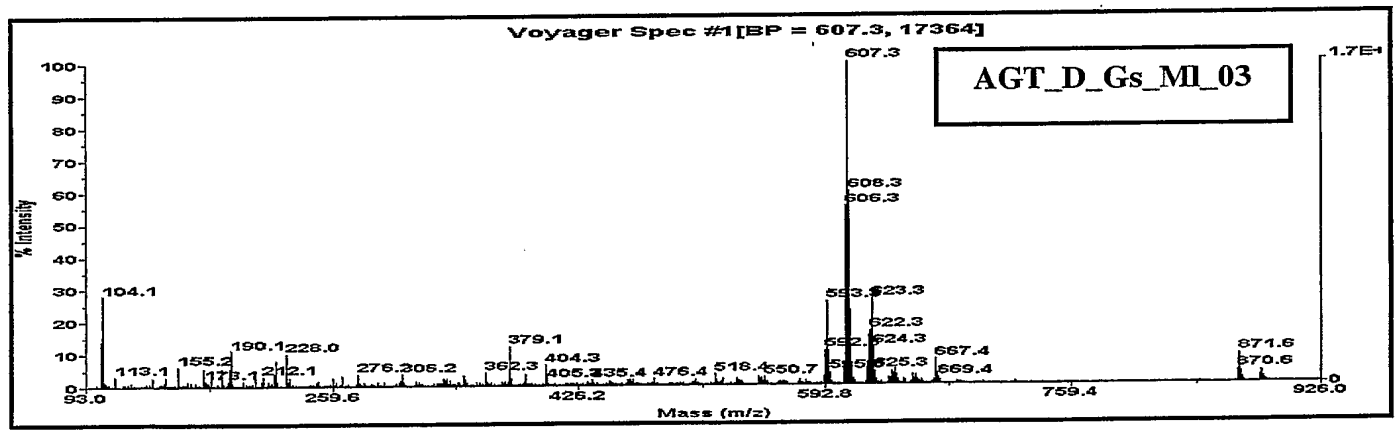
AGT_D_Ej_Se_03
% Intensity
Mass (m/z)
104.1
184.1
520.4
172.1
379.1
190.1
349.2
496.4
758.6
522.4
784.6
820.6
861.7
9.2E+

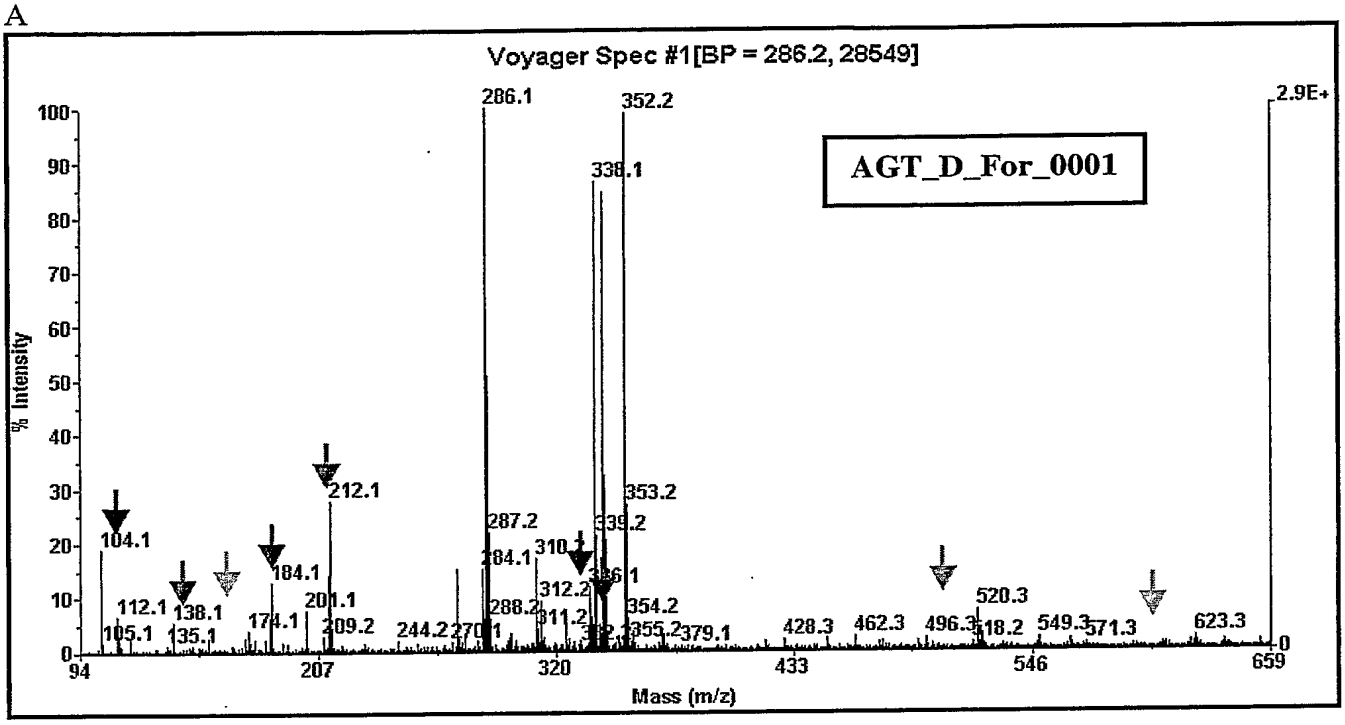
E

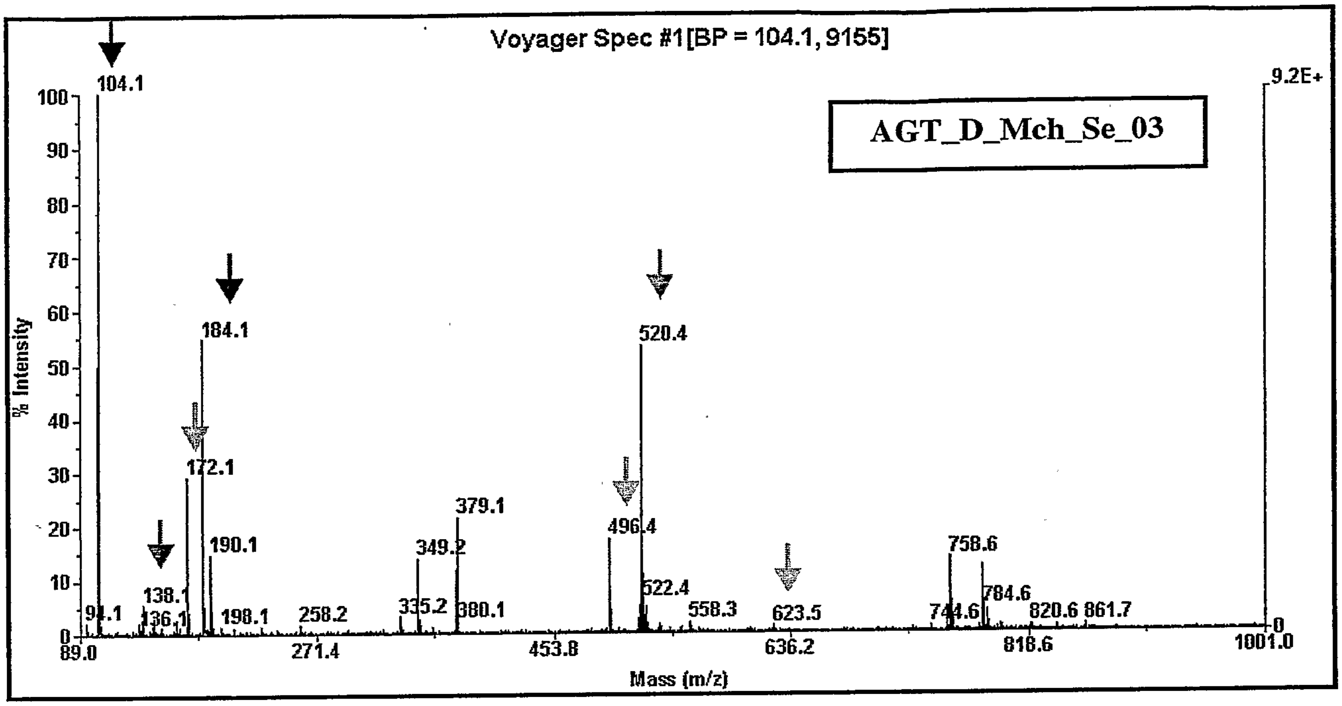

Voyager Spec #1[BP = 607.3, 17364]
AGT_D_Gs_MI_03
% Intensity
Mass (m/z)
607.3
608.3
506.3
104.1
623.3
622.3
624.3
379.1
190.1
228.0
404.3
667.4
871.6
870.6
1.7E+

F

Figure 2 (A-E): Representative mass spectra of the comparative mass spectrometric analysis between the chloroform fractions of the formulation, AGT_D_For_0001_03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_03, AGT_D_Ej_Se_03, AGT_D_Tfg_Se_03 and AGT_D_Gs_Ml_03.

A

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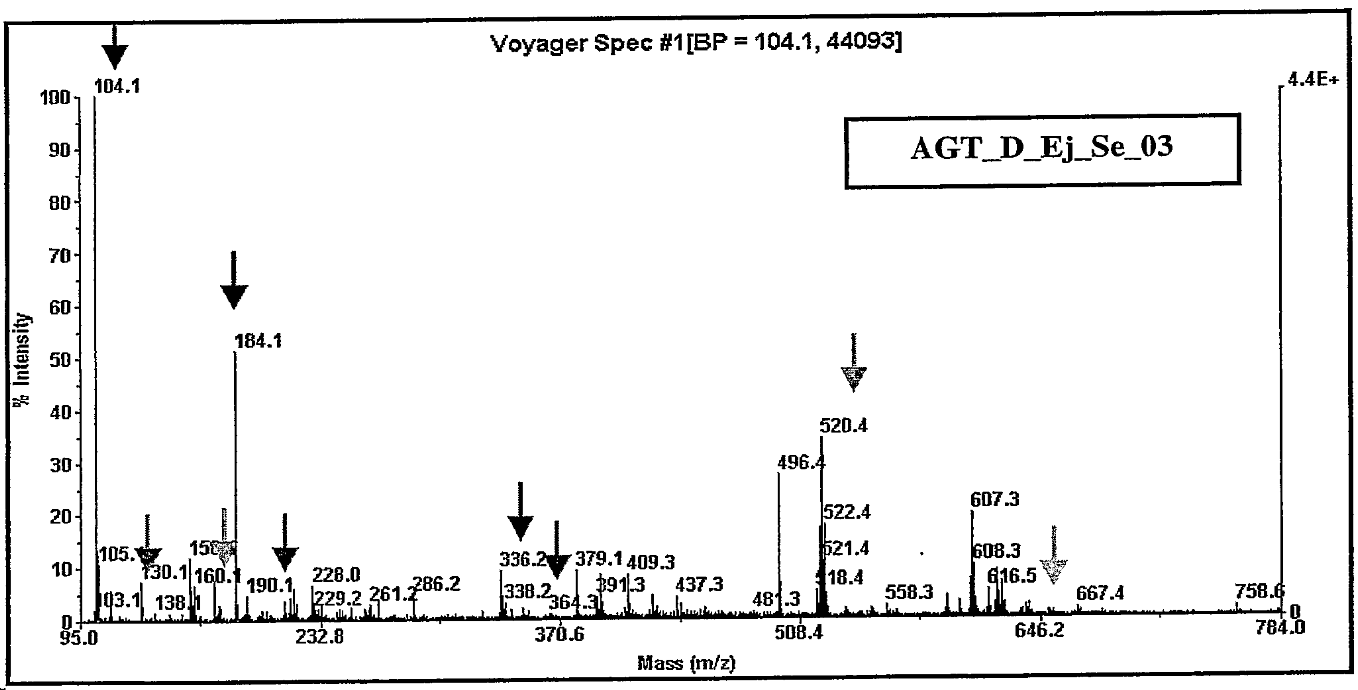
AGT_D_Mch_Se_03
% Intensity
Mass (m/z)
9.2E+

B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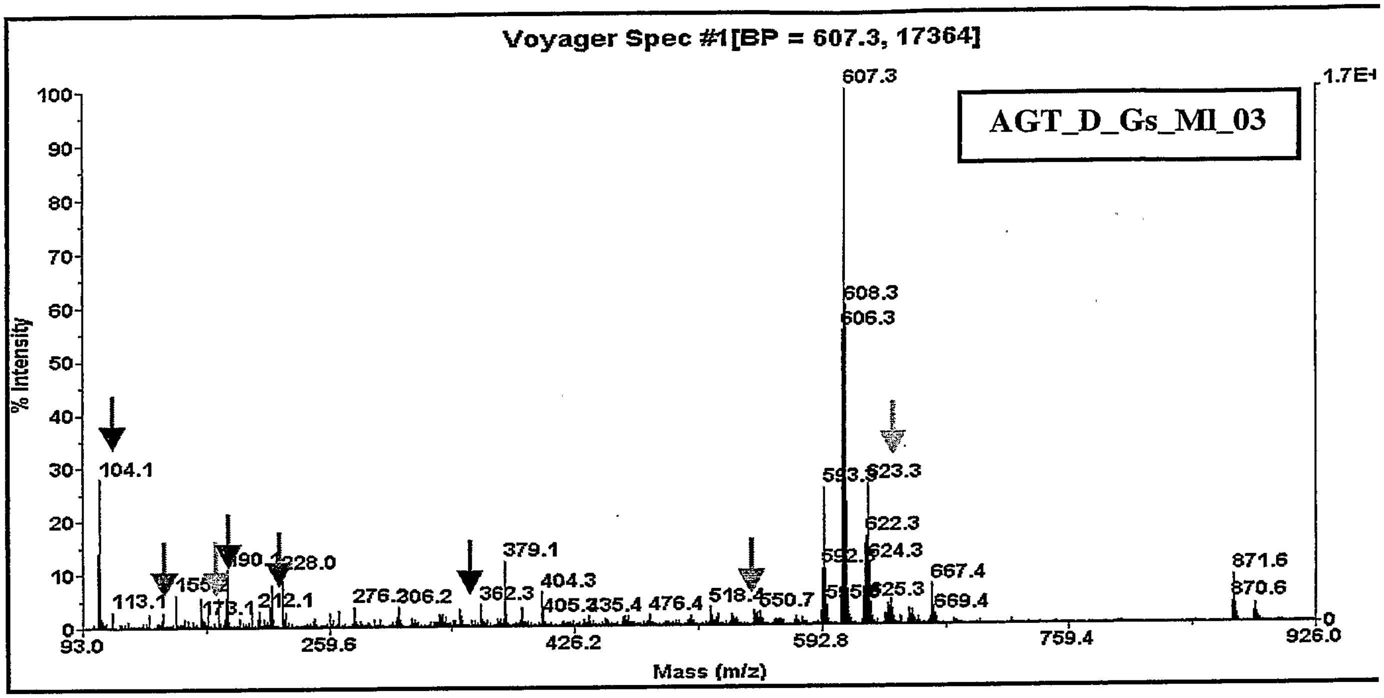
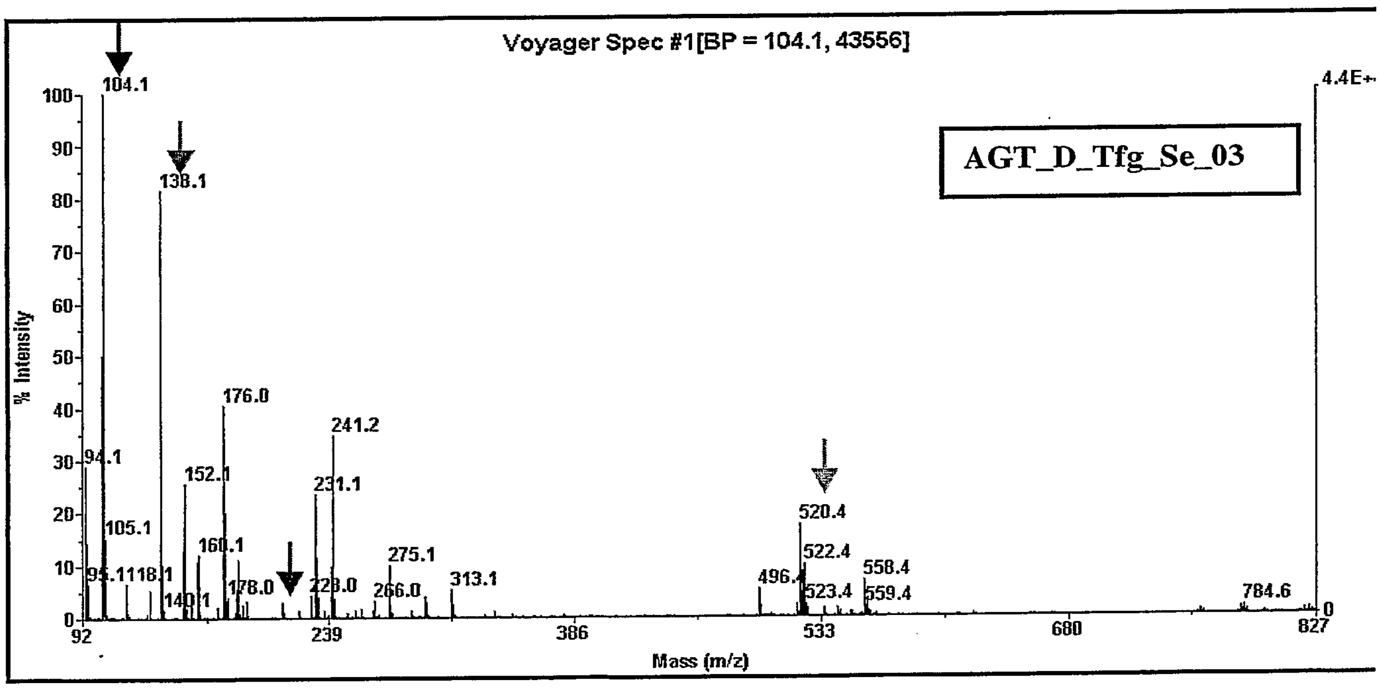
AGT_D_Ej_Se_03
% Intensity
Mass (m/z)
4.4E+

C


Voyager Spec #1[BP = 607.3, 17364]
AGT_D_Gs_Ml_03
% Intensity
Mass (m/z)
607.3
608.3
606.3
104.1
623.3
622.3
624.3
625.3
667.4
669.4
871.6
870.6
379.1
404.3
228.0
212.1
113.1
362.3
476.4
518.4
550.7
1.7E+
93.0
259.6
426.2
592.8
759.4
926.0

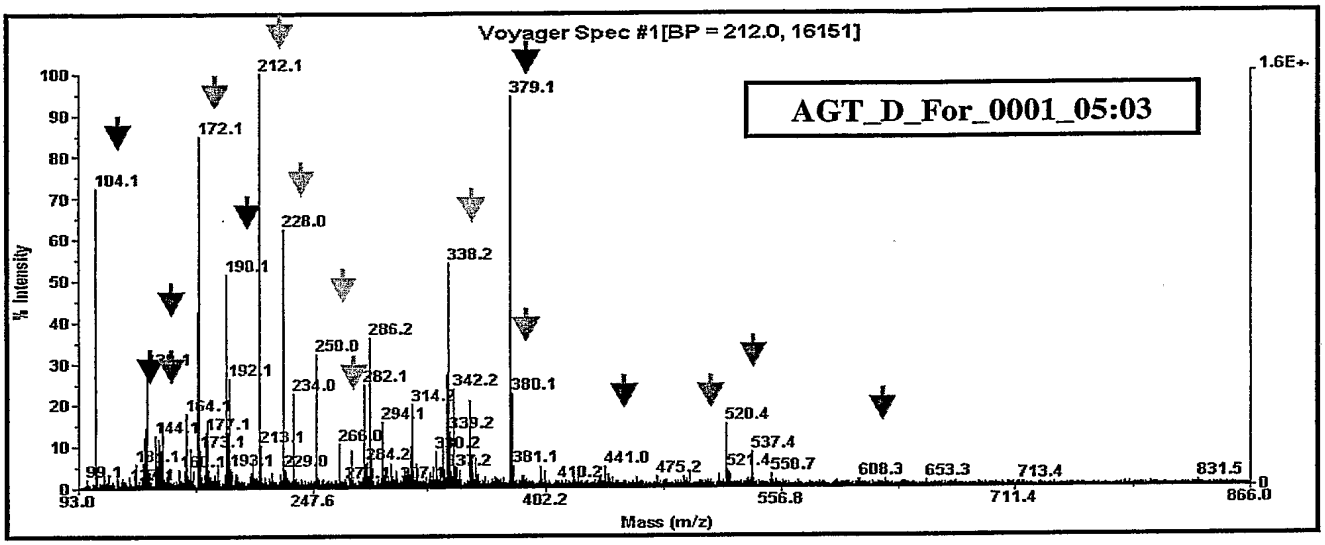
D


Voyager Spec #1[BP = 104.1, 43556]
AGT_D_Tfg_Se_03
% Intensity
Mass (m/z)
104.1
138.1
176.0
241.2
231.1
152.1
105.1
160.1
275.1
313.1
266.0
178.0
520.4
522.4
523.4
558.4
559.4
496.4
784.6
4.4E+
92
239
386
533
680
827

E

Figure 3 (A-E) Representative mass spectra of the comparative mass spectrometric analysis between the chloroform –methanol fractions of the formulation, AGT_D_For_0001_05:03 and the chloroform extracts of a few of its constituents, AGT_D_Mch_Se_)5:03, AGT_D_Ej_Se_)5:03, AGT_D_Tfg_Se_05:03 and AGT_D_Gs_Ml_05:03.

A.



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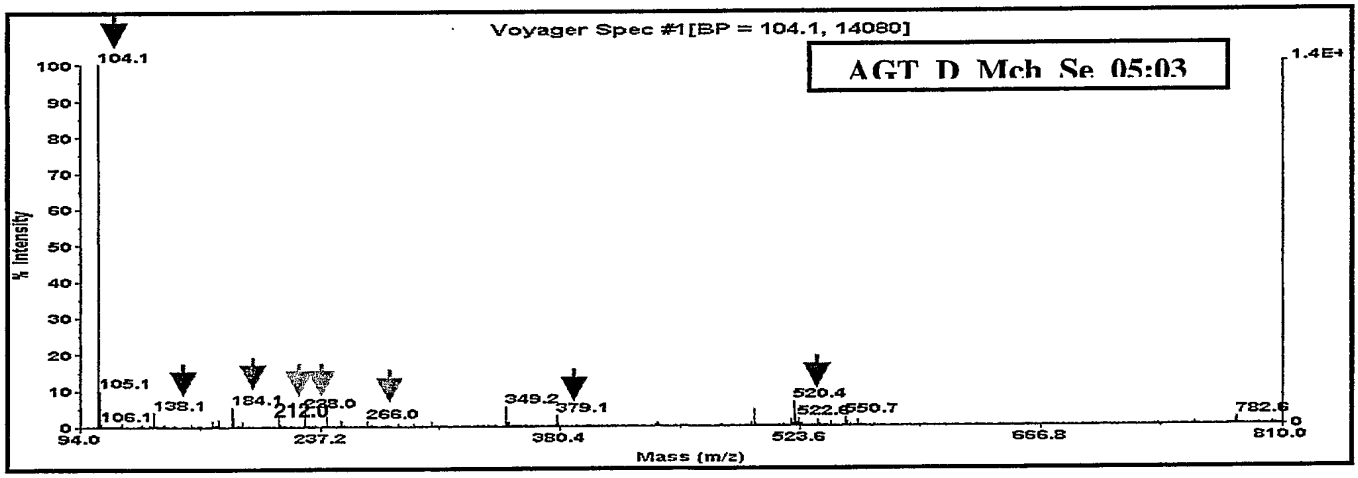

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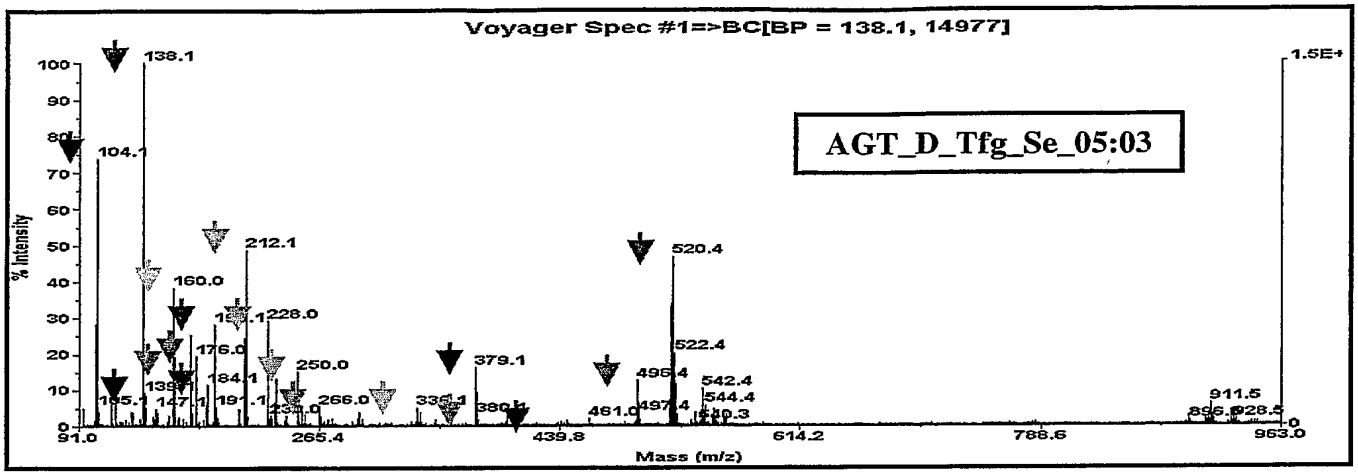
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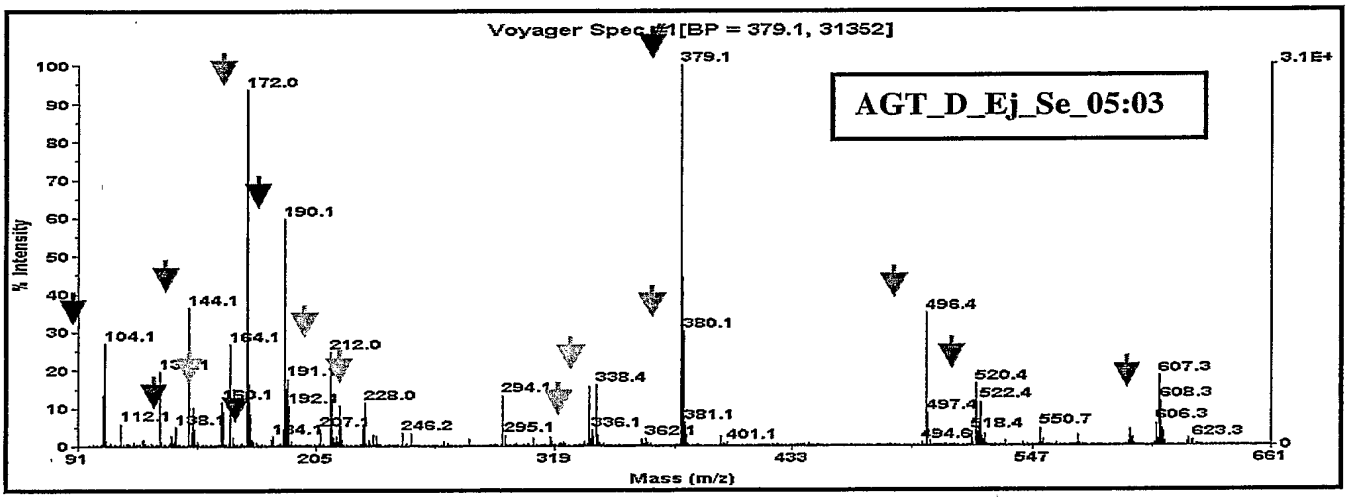
AGT_D_Tfg_Se_05:03
% Intensity
Mass (m/z)
138.1
104.1
212.1
160.0
520.4
228.0
176.0
250.0
379.1
522.4
542.4
544.4
911.5
91.0
265.4
439.8
614.2
788.6
963.0

D


Voyager Spec #1[BP = 379.1, 31352]
AGT_D_Ej_Se_05:03
% Intensity
Mass (m/z)
379.1
172.0
190.1
144.1
104.1
164.1
212.0
380.1
496.4
520.4
522.4
607.3
608.3
606.3
623.3
91
205
319
433
547
661

E

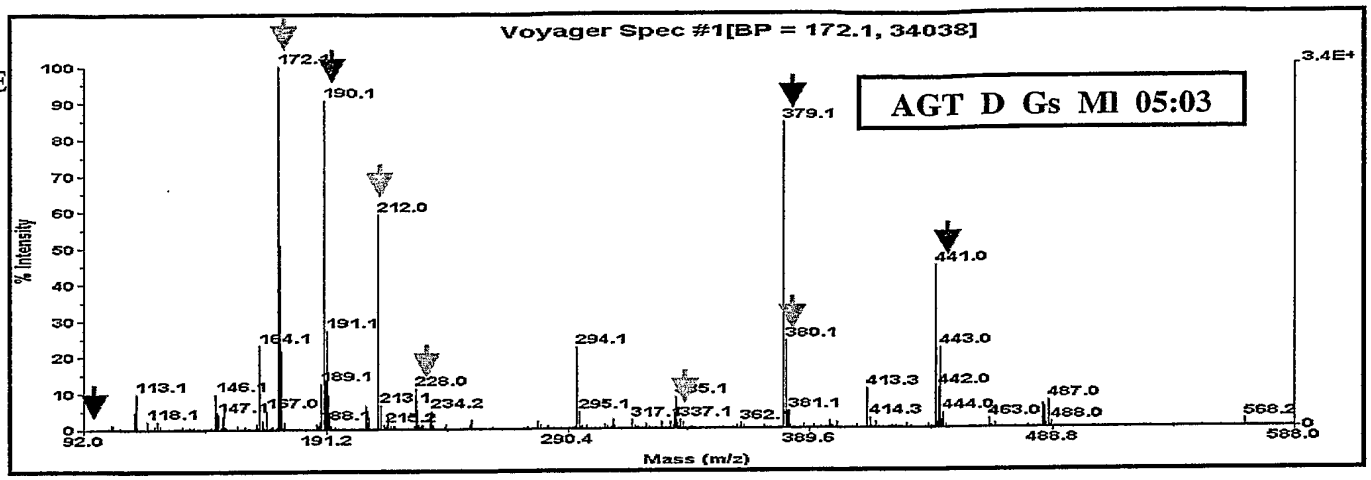
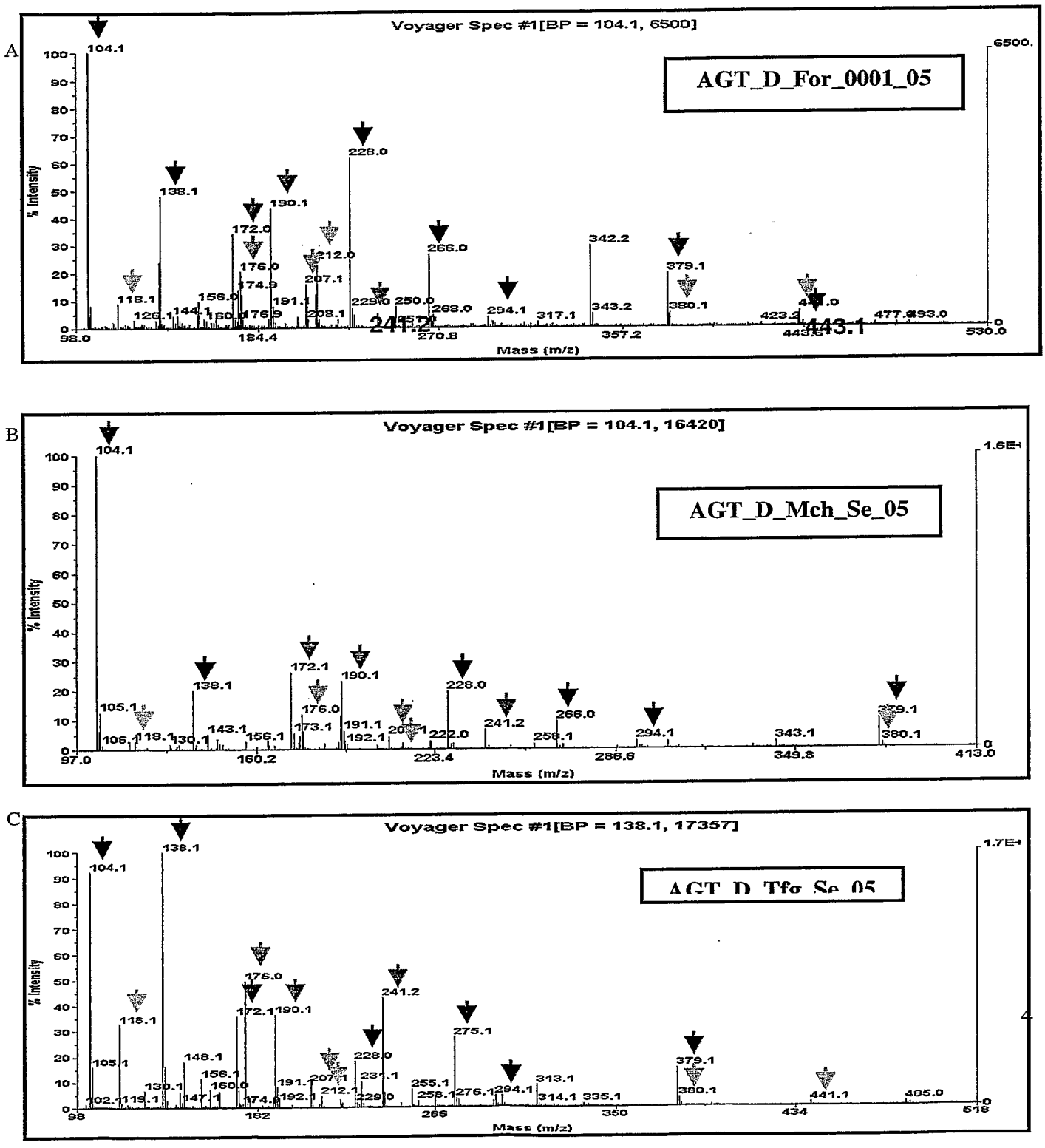
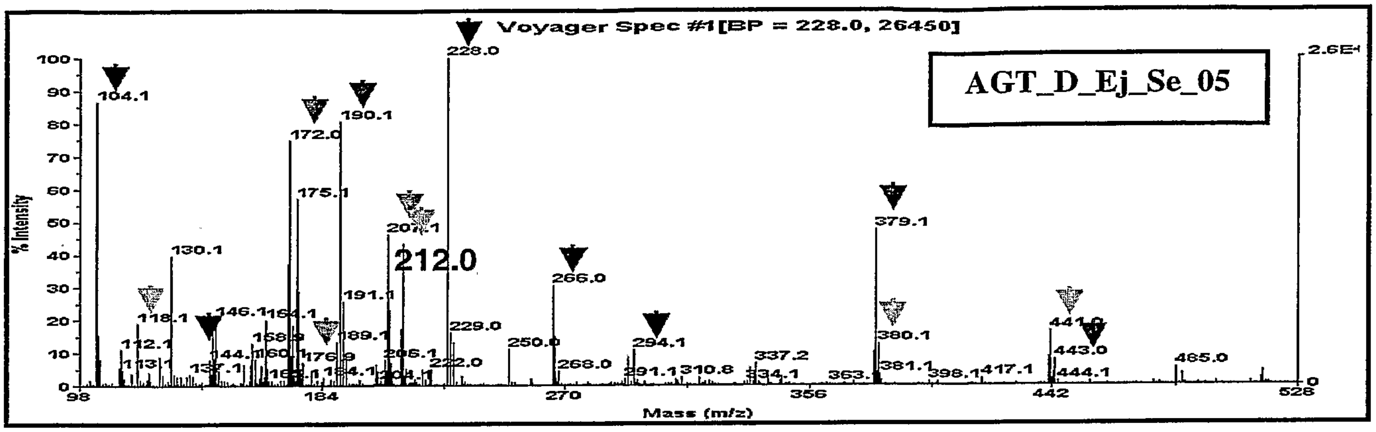

Voyager Spec #1[BP = 172.1, 34038]
AGT D Gs MI 05:03
% Intensity
Mass (m/z)
172.1
190.1
379.1
212.0
441.0
191.1
164.1
294.1
360.1
443.0
228.0
113.1
146.1
189.1
213.1
234.2
295.1
325.1
337.1
381.1
413.3
442.0
487.0
118.1
147.1
157.0
414.3
444.0
463.0
488.0
568.2
3.4E+
92.0
191.2
290.4
389.6
488.8
588.0

Figure 4 (A-E) Representative mass spectra of the comparative mass spectrometric analysis between the methanol fractions of the formulation, AGT_D_For_0001_05 and the methanol extracts of a few of its constituents, AGT_D_Mch_Se_05, AGT_D_Ej_Se_05, AGT_D_Tfg_Se_05 and AGT_D_Gs_Ml_05.

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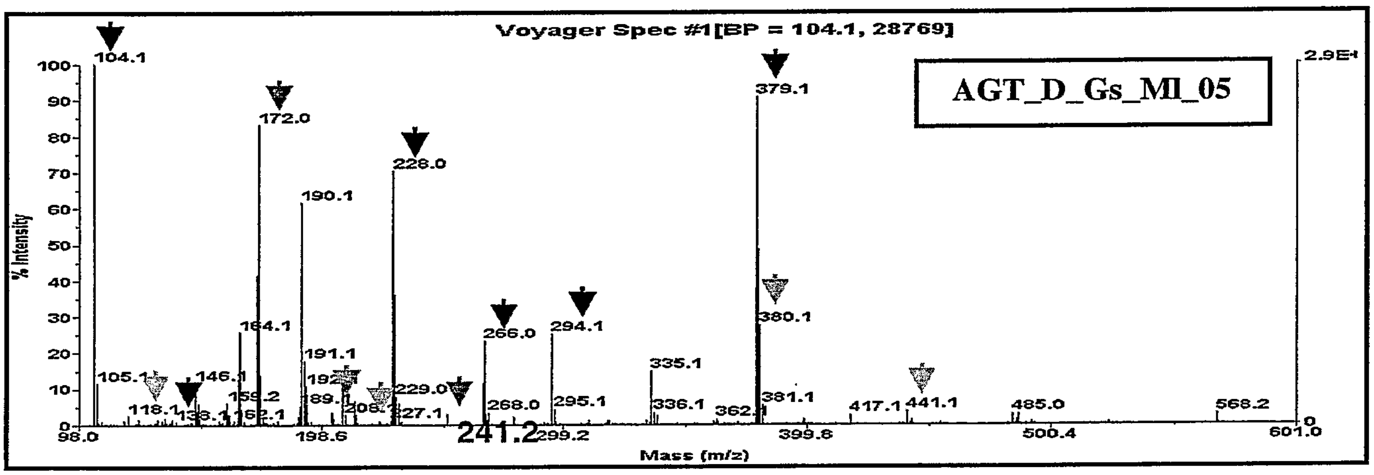
AGT_D_Ej_Se_05
% Intensity
Mass (m/z)
212.0

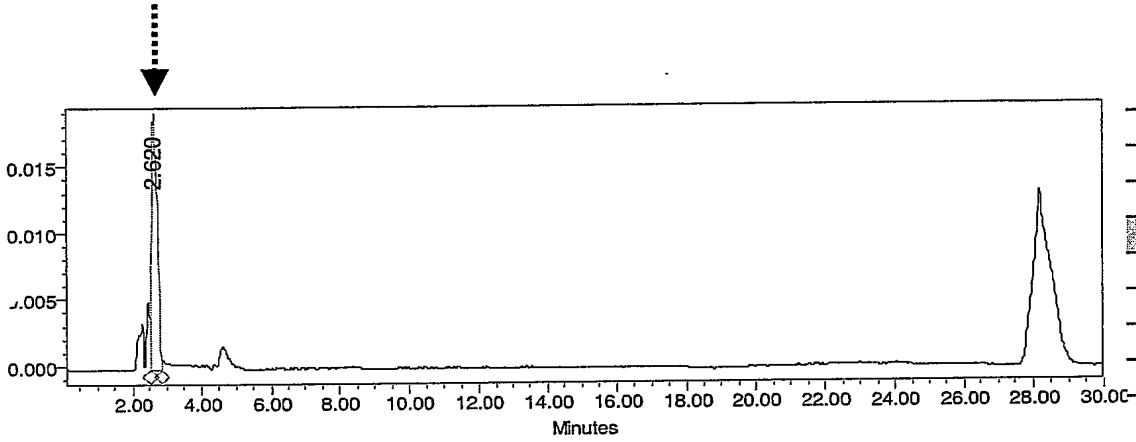
D


Voyager Spec #1[BP = 104.1, 28769]
AGT_D_Gs_Ml_05
% Intensity
Mass (m/z)
241.2

E

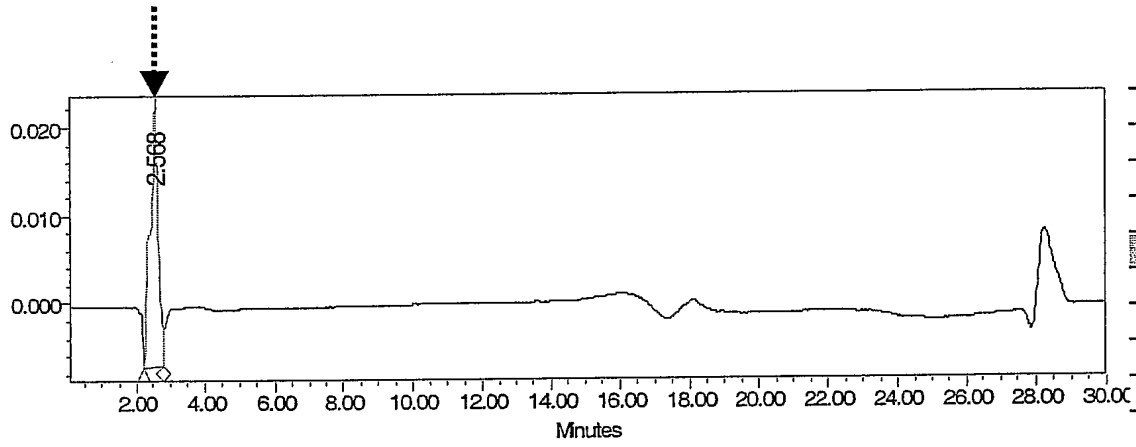
Fig 5: Chromatograms of extracts Ma_Le_Wa_01 and Ws_Ro_Wa_01 at selected wavelengths

A. Ma_Le_Wa_01 at 580 nm



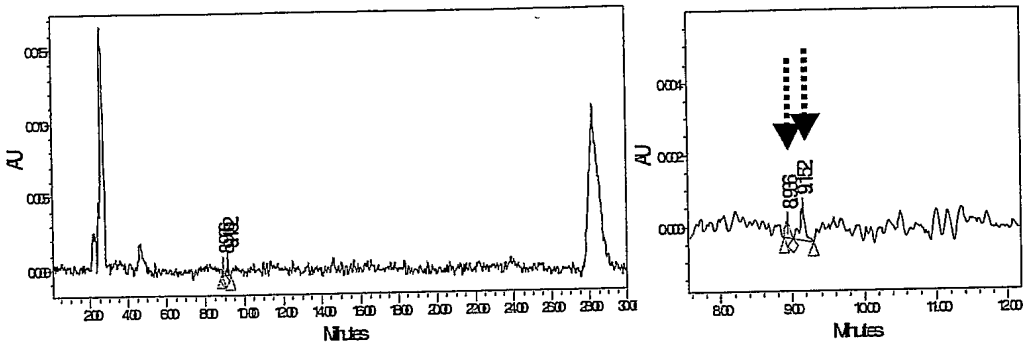
	R. Time	Lambda Max	Abs. M
1	0.107	646	0.00
2	2.073	201.9	1.76
3	2.620	485	0.00
4	21.014	353.6	0.00
5	23.429	190.1	0.0
6	23.813	190.1	0.42
7	28.177	190.1	0.96

B. Ws_Ro_Wa_01 at 580 nm



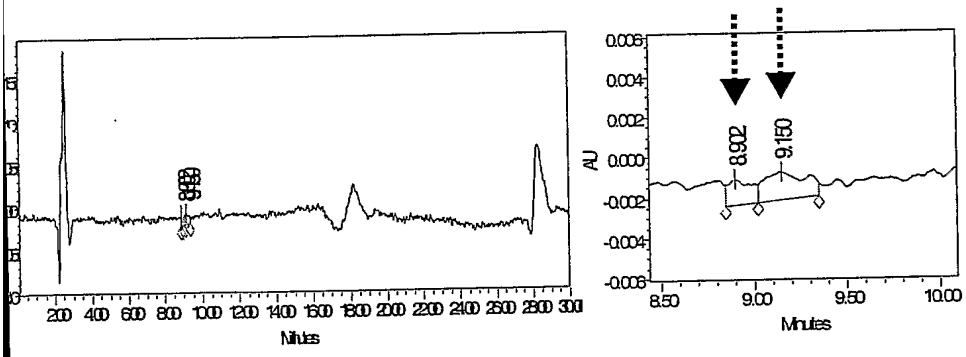
	R. Time	Lambda max	Abs. M
1	0.102	668.1	0.00
2	0.102	668.1	0.00
3	2.301	328.5	0.02
4	2.568	485	0.00
5	2.901	658.3	0.00
6	17.862	200.7	0.0
7	18.095	199.5	0.02
8	28.241	192.5	0.05

C. Ma_Le_Wa_01 at 650 nm



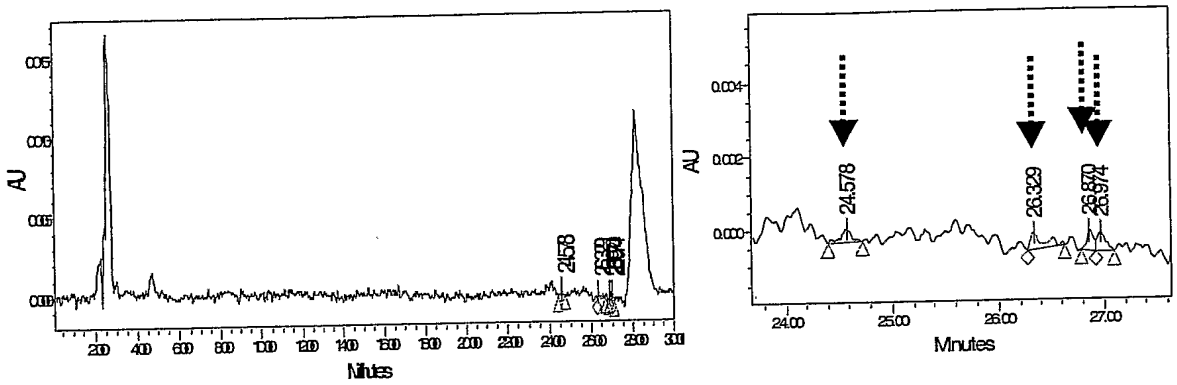
	R. Time	Lambda Max	Abs.
28	6.954	649.7	0.001
29	7.254	658.3	0.000
30	7.77	649.7	0.001
31	8.087	649.7	0.001
32	8.42	649.7	0.000
33	8.52	666.9	0.000
34	8.72	649.7	0.001
35	8.936	660.8	0.000
36	9.152	660.8	0.000
37	9.286	668.1	0.000
38	9.319	649.7	0.000
39	9.469	649.7	0.000
40	9.769	649.7	0.000
41	9.902	649.7	0.000
42	10.952	649.7	0.000
43	11.102	649.7	0.000
44	11.218	205.4	0.000
45	11.452	192.5	0.002
46	11.602	191.3	0.001

D. Ws_Ro_Wa_01 at 650 nm



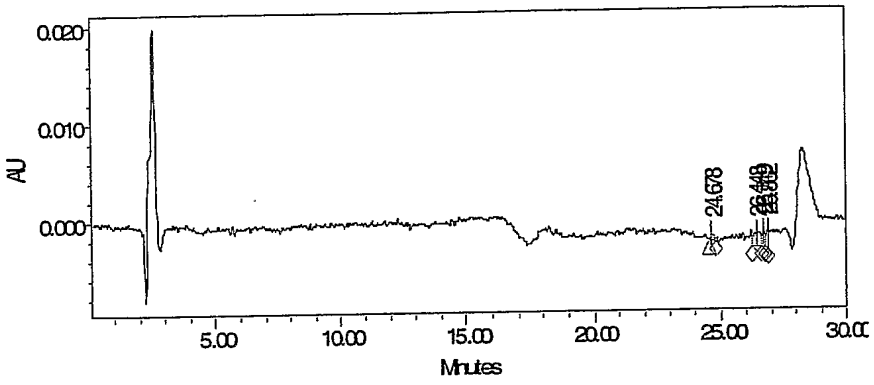
	R. Time	Lambda max	Abs. Max
38	7.649	697.5	0.00262
39	7.899	660.8	0.00248
40	8.182	660.8	0.00249
41	8.382	660.8	0.00344
42	8.532	660.8	0.00297
43	8.715	660.8	0.00326
44	8.902	660.8	0.0029
45	9.150	660.8	0.00265
46	9.265	651	0.00313
47	9.598	660.8	0.00313
48	9.715	660.8	0.00287
49	9.865	192.5	0.00876

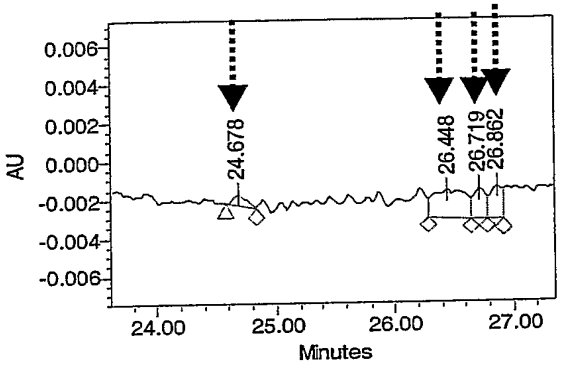
E. Ma_Le_Wa_01 at 690 nm



	R. Time	Lambda Max	Abs. Max
90	24.013	190.1	0.35506
91	24.212	190.1	0.41454
92	24.379	190.1	0.44123
93	24.579	190.1	0.45064
94	24.795	190.1	0.45579
95	24.912	190.1	0.44643
96	25.129	190.1	0.42456
97	25.279	190.1	0.40138
98	25.728	190.1	0.31581
99	25.828	190.1	0.29505
100	26.095	190.1	0.23558
101	26.329	190.1	0.20293
102	26.528	190.1	0.12837
103	26.870	190.1	0.05476
104	26.974	190.1	0.01977
105	27.161	192.5	0.07595
106	27.228	192.5	0.10762
109	28.56	190.1	0.0157
110	29.343	697.5	0.00093
111	29.693	651	0.0008

F. Ws_Ro_Wa_01 at 690 nm

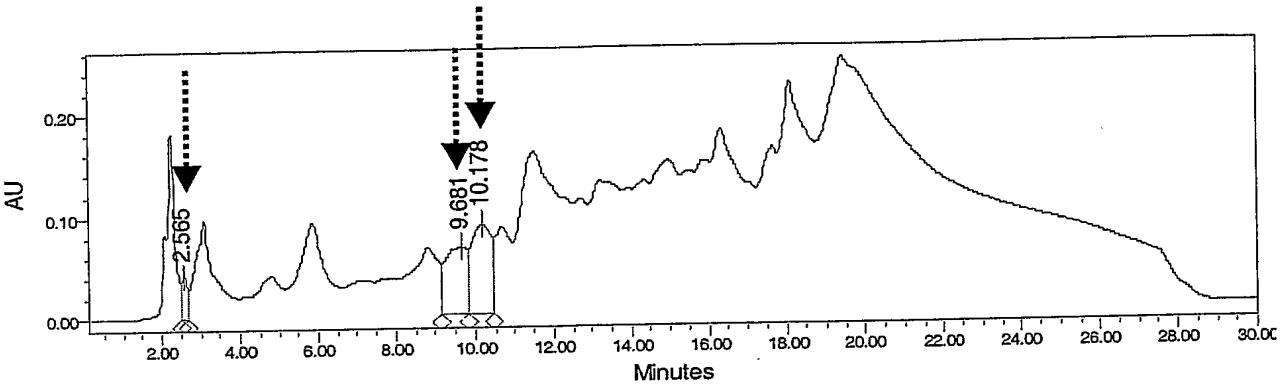




	R. Time	Lambda max	Abs. Max
88	23.276	199.5	0.01191
89	23.709	193.6	0.00354
90	23.909	192.5	0.00157
91	24.159	191.3	0.00458
92	24.678	190.1	0.00477
93	24.776	197.2	0.00349
94	24.875	194.8	0.00495
95	25.075	190.1	0.00559
96	25.159	194.8	0.00554
97	25.342	193.6	0.0023
98	25.775	194.8	0.00623
99	25.875	197.2	0.00387
100	26.058	194.8	0.00458
101	26.308	690.2	0.00113
102	26.448	190.1	0.00492
103	26.719	190.1	0.00528
104	26.862	190.1	0.00627
105	27.658	197.2	0.09525
106	28.224	192.5	0.04129
107	29.257	191.3	0.00285
109	29.624	190.1	0.0024
110	29.79	191.3	0.00104

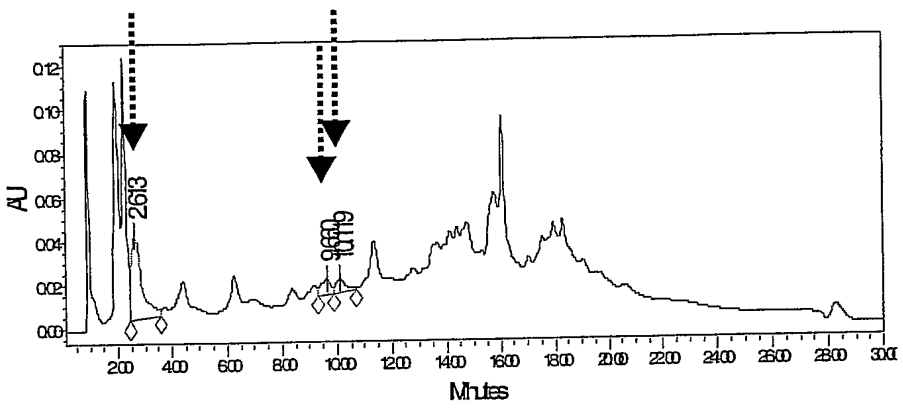
Fig 6: Chromatograms of extracts Cr_Ro_Et_01 and Ws_Ro_Et_01 at selected wavelengths

A. Cr_Ro_Et_01 at 270 nm



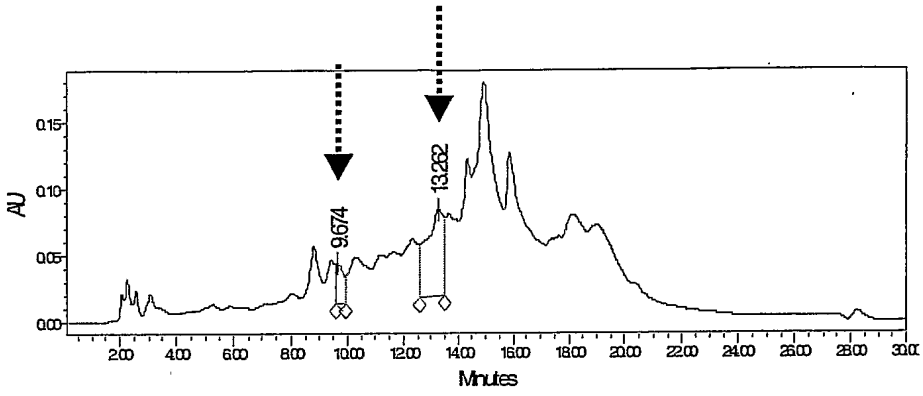
	R. Time	Lambda Max.	Abs. Max
1	2.087	199.5	0.50854
2	2.254	234.8	1.9173
3	2.565	192.5	0.33016
4	3.087	232.4	0.64575
5	4.22	194.8	0.11512
6	4.82	192.5	0.19056
7	5.87	192.5	0.4551
8	7.236	193.6	0.49654
9	7.736	193.6	0.59015
10	8.802	193.6	0.79979
11	9.681	193.6	0.83845
12	10.178	193.6	0.94701
13	10.685	194.8	0.98703
14	11.518	194.8	1.31397
15	12.285	194.8	1.24312
16	12.701	194.8	1.26801
17	13.218	194.8	1.35583
18	13.434	196	1.35745
19	13.951	196	1.39184
20	14.334	196	1.45647
21	14.967	196	1.52994
22	15.484	196	1.50795
23	15.851	197.2	1.55668
24	15.967	197.2	1.56793
25	16.317	197.2	1.62721
26	17.65	199.5	1.84688
27	18.1	199.5	2.01099
28	19.417	199.5	2.00576

B. Ws_Ro_Et_01 at 270 nm



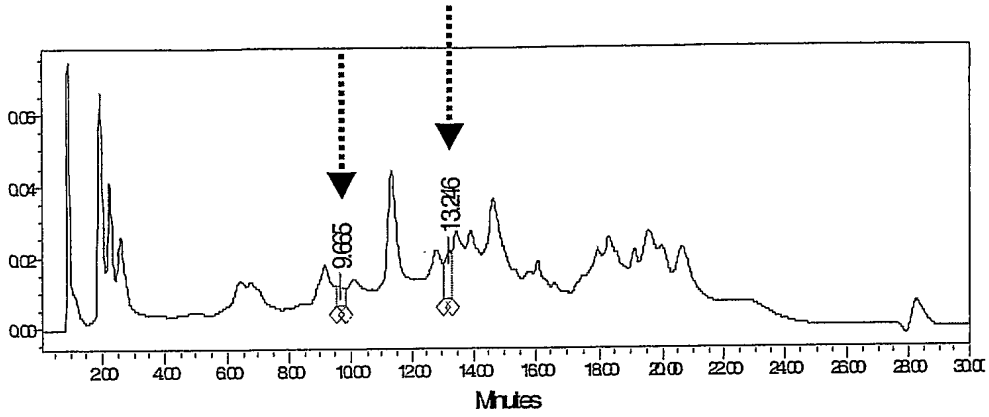
	R. Time	Lambda Max	Abs. Max
1	0.115	190.1	0.00028
2	0.948	197.2	0.41888
3	1.947	197.2	0.68039
4	2.231	193.6	0.79799
5	2.613	192.5	0.19402
6	3.73	200.7	0.01838
7	4.397	198.3	0.09547
8	6.247	194.8	0.18099
9	6.996	192.5	0.01873
10	8.379	194.8	0.08258
11	9.179	193.6	0.04649
12	9.660	193.6	0.0514
13	10.119	193.6	0.05152
14	10.696	193.6	0.03452
15	11.345	194.8	0.0667
16	11.929	192.5	0.02847
17	12.812	197.2	0.09257
18	13.295	196	0.14591
19	13.662	197.2	0.32274
20	13.912	197.2	0.26242
21	14.111	197.2	0.37587
22	14.378	200.7	0.95824
23	14.745	224.2	1.1217
24	15.328	197.2	0.41881
25	15.761	199.5	0.85325
26	16.078	197.2	0.63455
27	17.044	198.3	0.4413
28	17.561	200.7	0.57871
29	17.961	200.7	0.6471
30	18.294	200.7	0.72115
31	19.044	200.7	0.50734
32	19.644	200.7	0.38701
33	20.61	200.7	0.20326
34	28.242	192.5	0.05557

C. Cr_Ro_Et_01 at 330 nm



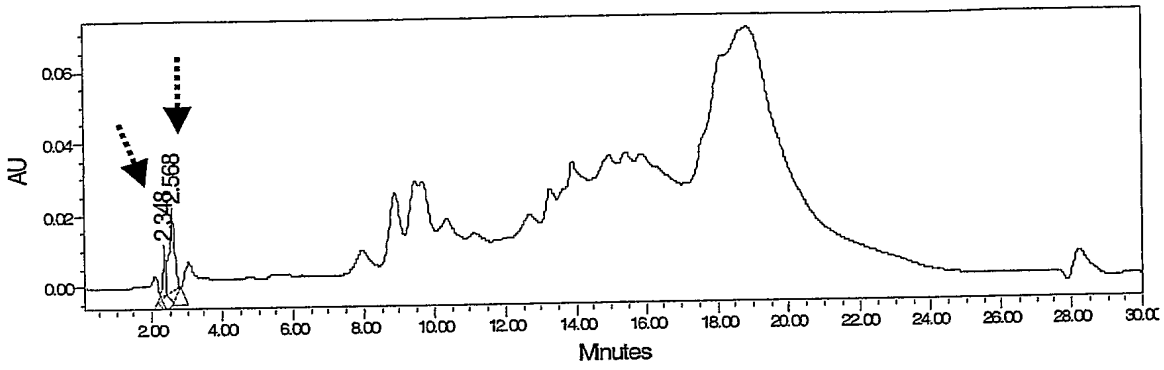
	R. Time	Lambda Max.	Abs. Max
1	2.07	200.7	0.4866
2	2.254	234.8	1.88548
3	2.57	192.5	0.21094
4	3.053	232.4	0.77904
5	3.387	205.4	0.10594
6	4.087	286.9	0.00364
7	4.353	290.4	0.00329
8	5.27	286.9	0.00784
9	5.853	246.6	0.18044
10	7.119	193.6	0.04334
11	8.036	193.6	0.09153
12	8.785	193.6	0.2405
13	9.435	193.6	0.20392
14	9.674	193.6	0.21604
15	10.335	193.6	0.29389
16	11.185	194.8	0.34762
17	11.668	194.8	0.50063
18	12.335	194.8	0.3846
19	13.262	194.8	0.4188
20	13.651	194.8	0.37805
21	14.334	196	0.36579
22	14.934	196	0.3696
23	15.851	196	0.29167
24	17.4	197.2	0.18577
25	17.667	201.9	0.31291
26	18.1	201.9	0.48327
27	19	199.5	0.17848
28	28.215	485	0.01591

D. Ws_Ro_Et_01 at 330 nm



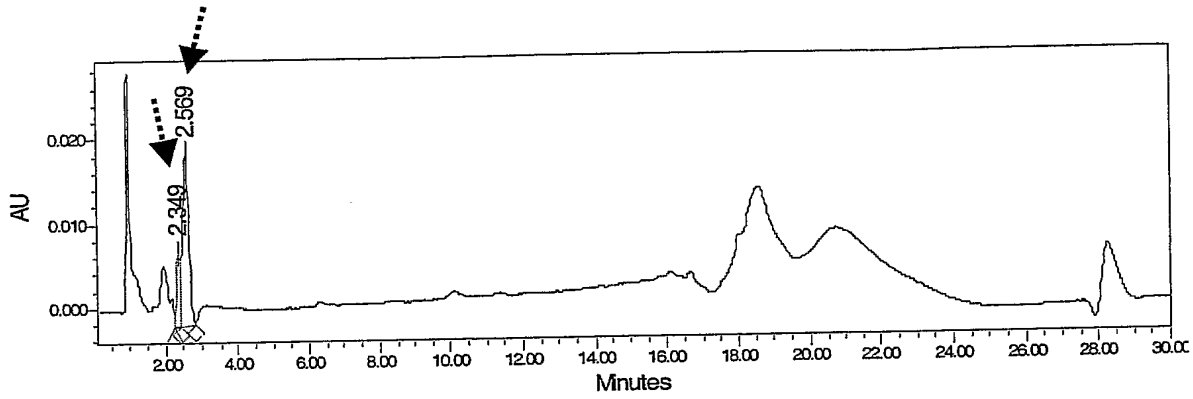
	R. Time	Lambda Max	Abs. Max
1	0.115	190.1	0.00028
2	0.948	197.2	0.40018
3	1.931	196	0.63299
4	2.247	193.6	0.77062
5	2.581	192.5	0.17303
6	6.43	192.5	0.04709
7	6.747	192.5	0.02341
8	8.413	194.8	0.08999
9	9.163	193.6	0.08332
10	9.665	193.6	0.10695
11	10.112	193.6	0.12405
12	11.329	194.8	0.19829
13	12.162	194.8	0.16632
14	12.812	194.8	0.2143
15	13.246	194.8	0.21948
16	13.462	194.8	0.22538
17	13.912	194.8	0.24628
18	14.628	217.1	1.0283
19	15.811	228.9	0.65343
20	16.094	231.3	0.47052
21	16.628	194.8	0.02992
22	17.477	201.9	0.33393
23	17.961	201.9	0.34255
24	18.311	201.9	0.48014
25	19.127	201.9	0.2821
26	19.594	200.7	0.23022
27	19.993	200.7	0.1926
28	20.643	200.7	0.11251
29	28.242	192.5	0.05651

E. Cr_Ro_Et_01 at 400 nm



	R. Time	Lambda Max.	Abs. Max
1	2.104	658.3	0.00939
2	2.348	485	0.02139
3	2.568	485	0.0388
4	3.037	233.6	0.45082
5	4.753	252.5	0.01205
6	5.42	420.8	0.00098
7	7.952	412.3	0.00628
8	8.869	193.6	0.08148
9	9.452	250.1	0.03225
10	9.669	389.4	0.02281
11	10.368	193.6	0.04742
12	11.135	193.6	0.01907
13	11.985	223	0.04917
14	12.685	194.8	0.00692
15	13.268	194.8	0.06038
16	13.901	196	0.03483
17	14.951	328.5	0.10475
18	15.384	328.5	0.02544
19	15.834	328.5	0.04568
20	18.217	203	0.64432
21	18.85	201.9	0.45036
22	28.215	485	0.01612

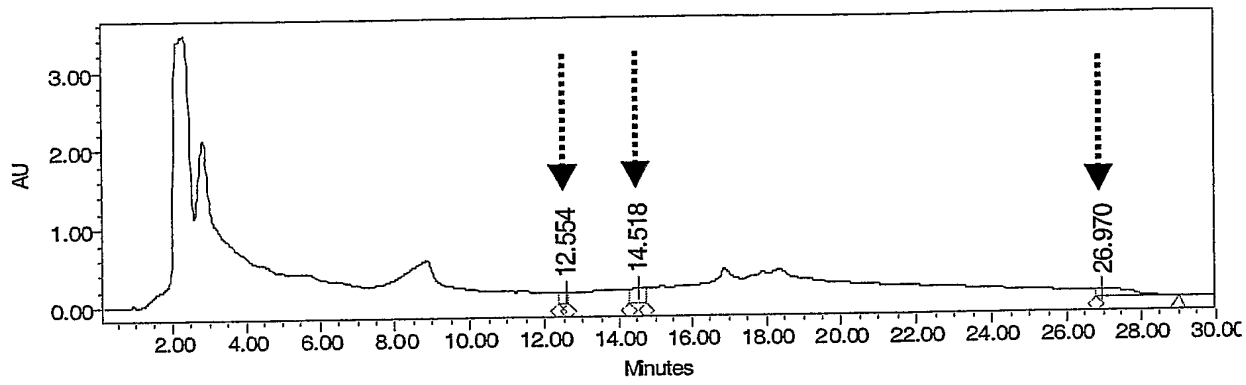
F. Ws_Ro_Et_01 at 400 nm



	R. Time	Lambda Max	Abs. Max
1	0.115	190.1	0.00028
2	0.948	199.5	0.32804
3	1.764	485	0.00682
4	1.947	206.6	0.16692
5	2.349	485	0.02023
6	2.569	485	0.04148
7	3.097	485	0.00834
8	6.263	226.5	0.03186
9	10.079	192.5	0.00543
10	15.811	230.1	0.52905
11	16.078	232.4	0.40994
12	16.644	357.2	0.00291
13	17.994	200.7	0.25105
14	18.544	200.7	0.26132
15	20.743	200.7	0.10022
16	28.242	192.5	0.05651

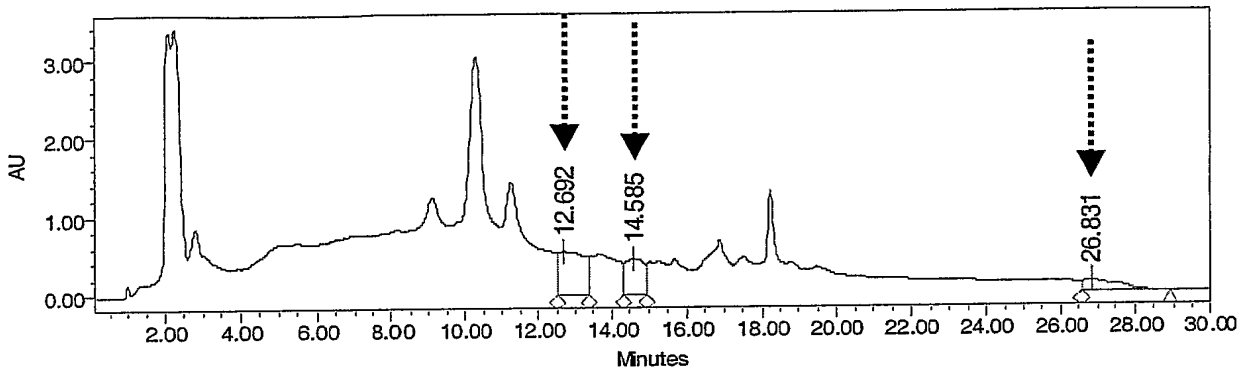
Fig 7: Chromatograms of extracts Ej_Se_Me_01 and Tt_Fr_Me_01 at selected wavelengths

A. Ej_Se_Me_01 at 210 nm



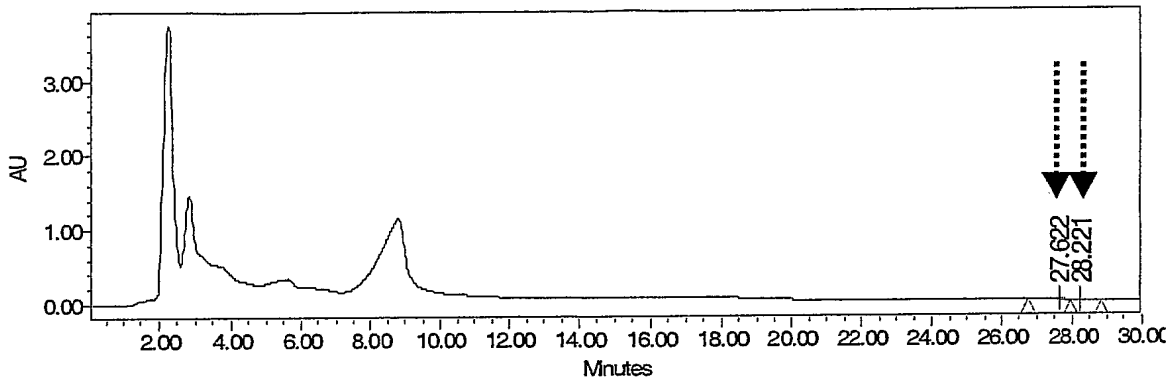
	R. Time	Lambda Max	Abs. Max
1	0.111	664.4	0.00017
2	0.928	197.2	0.0545
3	2.211	237.2	4.18161
4	2.311	231.3	4.08993
5	2.844	212.4	2.13403
6	5.727	192.5	0.58255
7	8.859	254.9	1.52953
8	10.509	193.6	0.83845
9	11.459	194.8	0.89837
10	12.059	194.8	0.93643
11	12.554	194.8	0.97463
12	13.625	194.8	1.09007
13	14.125	196	1.15174
14	14.518	196	1.20004
15	15.191	196	1.27047
16	15.858	197.2	1.32678
17	16.891	198.3	1.56607
18	17.608	198.3	1.54604
19	17.907	199.5	1.60032
20	18.357	199.5	1.62626
21	26.970	196	1.13028

B. Tt_Fr_Me_01 at 210 nm



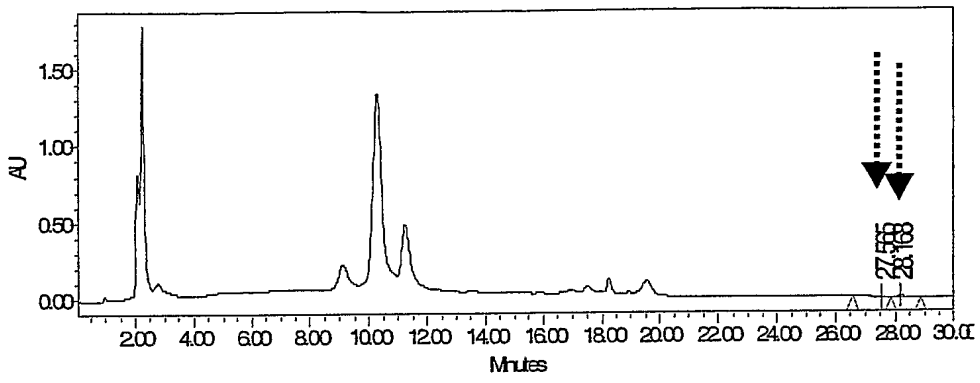
	R. Time	Lambda Max	Abs. Max
1	0.106	658.3	0.00036
2	0.106	658.3	0.00036
3	0.989	203	0.18095
4	1.389	204.2	0.17141
5	2.089	218.3	3.49797
6	2.239	232.4	3.89558
7	2.789	201.9	1.1211
8	3.939	204.2	0.41386
9	5.072	204.2	0.71949
10	5.505	204.2	0.75257
11	7.304	193.6	0.86758
12	8.154	193.6	0.9693
13	9.087	194.8	1.31876
14	10.287	210.1	3.03595
15	11.253	197.2	1.71047
16	12.692	194.8	1.22225
17	13.669	196	1.3187
18	14.585	196	1.37959
19	15.035	196	1.3731
20	15.285	197.2	1.40832
21	15.668	197.2	1.4549
22	16.901	199.5	1.69147
23	17.551	199.5	1.62387
24	18.234	204.2	2.24881
25	18.784	199.5	1.59787
26	19.201	199.5	1.52529
27	19.467	198.3	1.57611
28	21.317	198.3	1.40338
29	26.832	196	1.18442

C. **Ej_Se_Me_01 at 270 nm**



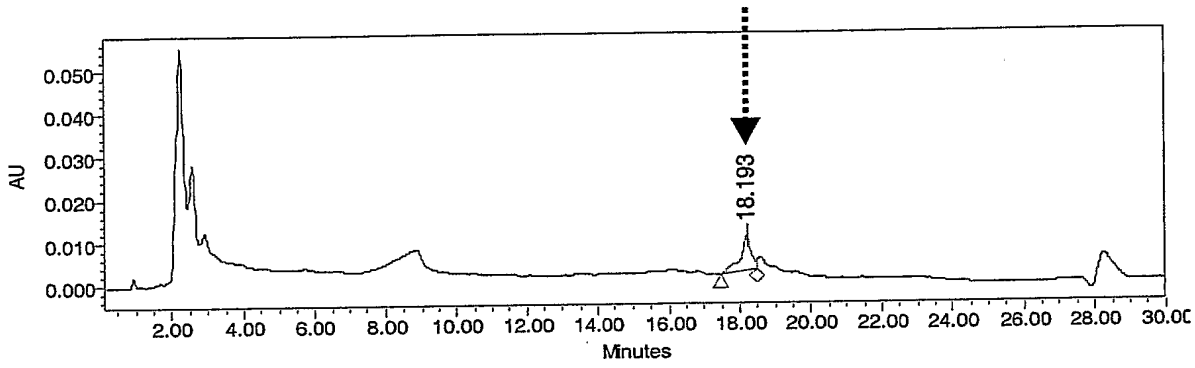
	R. Time	Lambda Max	Abs. Max
1	0.111	664.4	0.00017
2	0.928	200.7	0.04692
3	2.278	237.2	4.24142
4	2.844	212.4	2.11013
5	5.643	191.3	0.3677
6	6.26	191.3	0.30824
7	8.809	254.9	1.52352
8	10.526	192.5	0.20265
9	12.842	193.6	0.10449
10	13.408	193.6	0.09651
11	14.158	193.6	0.07521
12	14.642	193.6	0.06469
13	15.158	194.8	0.03509
14	15.525	193.6	0.01212
15	16.358	198.3	0.08989
16	16.558	199.5	0.14244
17	16.924	199.5	0.33248
18	17.757	200.7	0.35602
19	18.207	201.9	0.45986
20	27.622	197.2	0.24078
21	28.221	192.5	0.04592

D. Tt_Fr_Me_01 at 270 nm



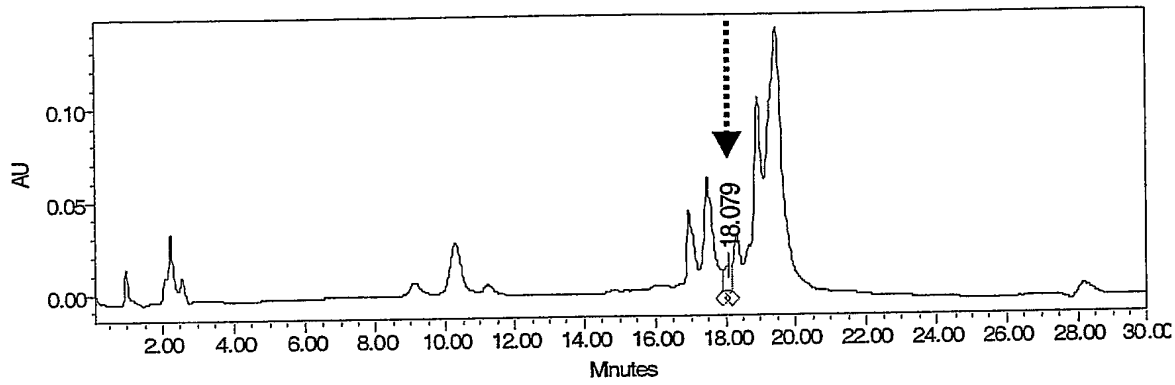
	R. Time	Lambda Max	Abs. Max
1	0.106	658.3	0.00036
2	0.106	658.3	0.00036
3	0.973	204.2	0.17422
4	1.389	206.6	0.15894
5	2.072	218.3	3.49477
6	2.239	232.4	3.89256
7	2.772	203	1.04068
8	3.939	206.6	0.34894
9	5.372	206.6	0.64765
10	6.788	207.7	0.71451
11	7.288	207.7	0.7341
12	7.721	207.7	0.7411
13	8.137	207.7	0.78696
14	8.521	207.7	0.79222
15	9.104	207.7	1.1811
16	10.287	210.1	2.94738
17	11.236	207.7	1.39175
18	12.269	193.6	0.58125
19	12.869	194.8	0.56012
20	13.486	194.8	0.53587
21	13.986	194.8	0.51658
22	14.485	194.8	0.50606
23	14.702	194.8	0.53125
24	15.185	196	0.46804
25	15.469	196	0.44948
26	15.902	196	0.42074
27	16.935	203	0.74559
28	17.468	200.7	0.50841
29	18.234	205.4	1.81022
30	18.634	201.9	0.51603
31	18.917	201.9	0.4448
32	19.534	409.9	0.38359
33	27.565	197.2	0.21209
34	28.165	192.5	0.04792

E. Ej_Se_Me_01 at 430 nm



	R. Time	Lambda Max	Abs. Max
1	0.111	664.4	0.00017
2	0.928	196	0.0514
3	1.661	223	0.00569
4	2.244	240.7	4.02357
5	2.561	213.6	0.71934
6	2.911	212.4	1.25696
7	8.859	254.9	1.24692
8	18.193	201.9	0.20084
9	18.574	203	0.12088
10	28.239	192.5	0.05227

F. Tt_Fr_Me_01 at 430 nm



	R. Time	Lambda Max	Abs. Max
1	0.106	658.3	0.00036
2	0.106	658.3	0.00036
3	0.973	203	0.14779
4	1.789	658.3	0.00616
5	2.239	239.5	3.69315
6	2.572	658.3	0.02939
7	2.905	658.3	0.00356
8	9.104	210.1	0.41005
9	10.287	210.1	2.28091
10	11.236	204.2	0.80513
11	13.186	191.3	0.00125
12	14.835	407.5	0.00123
13	16.152	437.7	0.00304
14	16.935	201.9	0.60222
15	17.451	200.7	0.38
16	18.079	201.9	0.44026
17	18.301	204.2	1.26276
18	18.667	201.9	0.39938
19	18.917	201.9	0.34766
20	19.451	409.9	0.45206
21	28.165	192.5	0.04642